Land Disposal Of
Waste Lubricating Oil

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ABSTRACT

Investigations to ascertain the factors and processes affecting the disappearance of waste lubricating oil from soil indicated that under field conditions at Timaru, New Zealand, waste lubricating oil applied at rates of up to 224 t/ha was lost at rates of up to 9.1 t/ha/month as determined by gravimetry of soil extracts. Field and laboratory evidence suggest that oil losses due to surface run-off, sub-soil leaching and evaporation were small and that oil recovery rate was constant for the duration of the field experiment. Increased microbial activity of the oiled soils, the ability of bacteria isolated from such soils to grow rapidly (as determined by large increases in bacterial numbers) in the presence of pure paraffinic oil as a sole carbon source and evidence for immobilization of nitrogen and phosphorus in the oiled soil suggested that microbial action was at least partly responsible for the observed losses.

The pattern and increased rates of respiration following addition of pure paraffinic oil to Timaru silt loam under laboratory conditions was evidence that at least part of the observed increase in carbon dioxide production was due to decomposition of the added hydrocarbons rather than mineralization of native soil organic matter. A corresponding decrease in the amount and apparent altered composition of oil present in the treated

soil compared with unaltered biologically inactive soil, as determined by gas chromatographic analysis of soil extracts, was considered to indicate that the oil had been modified chemically by biological processes.

Average rate of oil disappearance (over 18 months) under field conditions increased with increased oil application rate, a pattern which was repeated for laboratory respiration experiments with Timaru silt loam treated with pure paraffinic oil. Repeated cultivation of the oiled soils increased the rate of disappearance of waste lubricating oil at all application rates and the benefit was most marked for 56 and 224 t/ha oil application rates.

Evidence for increased rates of oil decomposition in the presence of added nutrients was provided by an increase in the average rate of oil disappearance following the application of nitrogen to oiled field plots, the lower exchangeable NO_3, NO_2 and NH_3 content of the oiled soils 20 months after oil application, evidence for phosphorus immobilization in the oiled soil and increased inferred average rates of oil mineralization of pure paraffinic oil mixed with Timaru silt loam amended with nitrogen and phosphorus.

Results obtained for laboratory soil respiration experiments provided evidence that oil mineralization rates were increased following a second application of oil. Other evidence obtained from soil respiration experiments suggested that the rate of disappearance of waste

lubricating oil from Timaru soil was not severely affected by the lead content of the oil. Repeated cultivation of the oiled soils increased the rate of disappearance of waste lubricating oil at all application rates and the benefit was most marked for 56 and 224 t/ha oil application rates. Production of volatiles from unoiled soils was stimulated by the presence of oil. Biological properties of the oiled soil as determined by the shoot dry matter yield of *Lolium multiflorum* Lam. var. Westerwolds "Grasslands Tama" were almost completely restored after twenty months further suggesting biochemical/chemical alteration of the oil. Lead analysis of the plant shoots after the same period indicated increased lead accumulation compared to that of the control. Maximum increase was obtained for 56 t/ha oiled plots while no significant increase was obtained for 224 t/ha suggesting that the higher concentration of oil residues may have suppressed lead uptake.

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CHAPTER I

LITERATURE REVIEW

Public awareness of the problems of oil pollution at sea and on land has been increased in recent years by the large numbers of oil tanker accidents at sea and by the indiscriminate dumping of waste oil on land.

Wardley-Smith (1976) has estimated that a total of 3.6 million tonnes of oil is discharged annually into the ocean from marine operational losses, accidental discharges, offshore production and oil seepage and land based discharges. Of this amount, an estimated 1.3 million tonnes or 36 percent is considered to originate from land based discharges including that of waste lubricating oils. A study by the U.S. National Academy of Sciences (1975) suggests that the total amount including that from atmospheric and surface runoff may be in excess of 6 million tonnes. Forty percent or 2.6 million tonnes was considered to result from land based discharges.

Use of mineral oils to lubricate internal combustion engines has led to there being considerable quantities of used lubricating oil for disposal or reuse. It has been estimated (Anon. 1972) that in the United States alone, 2.27×10^9 ℓ or in excess of 2 million tonnes of automobile oils are in need of disposal each year.

In a more recent study, (FAO 1977) it was estimated 5×10^6 tonnes of petroleum hydrocarbons enter the oceans

of the world from both onshore and offshore sources. Results of an investigation by the National Academy of Sciences (1975) suggested that more than 50 percent of the oil originated from land based activity. Technological and collection costs often make it uneconomic to recycle oil and Kelsall (1973) has estimated that between only 5 and 6 percent of the total engine oil sold in Australia and the United States respectively is reused. The balance, because it requires disposal has been termed waste.

In some areas of New Zealand, disposal of waste oils from internal combustion engines has become a problem. The possibility of waste oil disposal by land spreading has been considered by the New Zealand city of Timaru because of hazards associated with tipping and burning. Reports in the literature on waste oil disposal by this means with few exceptions, concern only crude oil refining wastes and residues from tankers and drilling operations. It was therefore decided that before the practice could be advocated its safety would need to be demonstrated.

Lubricating oils are highly refined, principally paraffinic fractions of compositionally more complex crude oils which contain mixtures of hydrocarbons of varying molecular weight and structure comprised of three main chemical groups, paraffinic, napthenic and aromatic.

Research into ways in which oil is degraded (and

otherwise dissipated in the environment) has been largely concerned with crude petroleum in the marine environment. Processes which have been observed to be responsible include emulsification, sedimentation, evaporation, solution and chemical and biological degradation. With the exception of the first two, it is conceivable that these processes contribute to the degradation of oil in soil.

Evaporation is the process by which low to medium molecular weight components of relatively low boiling point are volatolized into the atmosphere. The rate of evaporation is a function of the vapour pressure of each component of the oil, and its concentration, surface area and thickness of the oil film, wind and temperature (FAO 1977). The little available information concerning evaporation of hydrocarbons has been reviewed by the FAO (1977).

The percentage of components lost to atmosphere in aquatic environments has been found to correlate well with carbon number. All hydrocarbons containing approximately 13 carbons or less are subject to great losses a few days after oil has been spilt at sea whilst heavier fractions evaporate more slowly. Evaporation therefore selectively depletes the lower boiling components of the oil thereby increasing the specific gravity.

Using gas chromatography, Regnier & Scott (1975) investigated evaporation of the alkane fraction of a

fuel oil cut between 130°C and 280°C. Three millimetre thick layers of oil floating on a water column were subjected to a constant wind speed of 21 km/h and temperatures of 5°C, 10°C, 20°C and 30°C. These experiments showed that for a particular alkane, the evaporation rate constant increased with temperature and that for a given temperature, the rate constant decreased with increasing molecular weight. Evaporation rate constants determined for alkanes under the temperatures investigated, correlated well with evaporation of the oil. Kinney (1969) also studied evaporation of oil and concluded that components with vapour pressures lower than n-octadecane will not evaporate significantly under 'normal' marine conditions while those with higher vapour pressure would not be expected to persist long in the open marine environment.

Solution is the physical process by which low molecular weight hydrocarbons as well as some of the more polar hydrocarbons are lost by oil to water. The rate of this process at sea is governed by wind, air, sea state and by the properties of the petroleum including chemical composition, specific gravity, viscosity, pour-point, surface tension and solubility (FAO 1977).

The solubilities of the lighter n-paraffins in fresh water are roughly proportional to their vapour pressures and the logarithms of the vapour pressure of n-paraffins in the range C_5 to C_{16} are proportional to their carbon

number (Parker et al. 1970). Extrapolating this data, Parker et al. obtained order of magnitude solubilities in fresh water of 1 x 10^{-4} to 2 x 10^{-4} g/m^3, 6 x 10^{-4} to 10^{-8} g/m^3, 10^{-7} to 10^{-14} g/m^3 and 10^{-14} g/m^3 for kerosine (C_{10} - C_{17}), gas oil (C_{16} - C_{25}), lubricating oil (C_{23} - C_{37}) and bitumen (> C_{37}) fractions respectively. Differences in solubility of hydrocarbons other than n-paraffins were neglected. Solution in fresh water of lubricating oil and bitumen fractions was considered negligible.

The solubility of several n-paraffins of carbon numbers C_{12} to C_{26} was investigated by Sutton and Calder (1974). An amount, 175 mg, of each hydrocarbon was equilibrated with 700 ml of sea and fresh water in a flask experiment. Paraffins were less soluble in salt than fresh water for which solubilities at 25°C ranged from 2.9 ppb for dodecane (C = 12) to 1.7 ppb for hexacosane (C = 26). Anderson (1974) compared oil-water dispersion and water soluble fractions of various crude and refined oils and obtained a larger water soluble fraction from the refined oils which had a greater concentration of di-and tri-aromatic hydrocarbons. By contrast, the crude oil water soluble fraction was rich in light aliphatics ranging from propane to isopentane and light aromatics.

Chemical alteration of crude oils has been reviewed by Parker et al. (1970). In the presence of air, the main chemical reactions undergone by oils were considered

to be oxidation reactions initiated either thermally or by the absorption of light. Oxidation of paraffins, non-conjugated olefins and many aromatics proceeds by a free radical chain reaction and is auto-catalytic, (Waters 1950). Once the reaction has been initiated, those which follow proceed at an increasing rate because the products of the reaction, hydroperoxides themselves decompose with the formation of more free radicals. The rate of reaction for a particular system is also governed by presence of inhibitors e.g. sulphur compounds, that act as terminators of chain reactions. The hydroxy compounds formed by decomposition of hydroperoxide may undergo further dehydrogenation and peroxidation to form aldehydes, ketones and carboxylic acids of low molecular weight. Products of higher molecular weight may also be formed. They result from radical-radical combination by condensation of aldehydes or ketones with phenols, or by esterification between alcohols and carboxylic acids. Parker et al. (1970) cited experiments in which thin films of oil immersed in aerated fresh water or sea water were exposed to light of wavelength shorter than 300 mm. Photo-oxidation in fresh water was found to be rapid and a large proportion of the oil was converted to acetic acid, sulphuric acid and carbon dioxide. The rate of photo-oxidation was considerably reduced in the presence of light having a wavelength interval within the range present in sunlight.

The chemical alteration of hydrocarbons by micro-

organisms has also been demonstrated by many workers. The earliest of these experiments was carried out by Sohngen (1906) who isolated and described a methane oxidizing bacterium *Bacillus methanicus*. Since this discovery, the chemical alteration of hydrocarbons by soil bacteria and actinomycetes and fungi has been demonstrated in mineral salts solution by a number of workers using single hydrocarbons and complex hydrocarbon substrates such as petroleum e.g. Imelik (1948), Azoulay et al. (1958), Cundell and Traxler (1973), Thijsse et al. (1958), Marr et al. (1961), Stevenson et al. (1962), Senez et al. (1963), Leadbetter & Foster (1960), Soli et al. (1972) Walker et al. (1974), Pritchard et al. (1975), Gibson (1968). Much of this work has been reviewed extensively by ZoBell, (1946, 1950), Foster (1962), Kallio et al.(1969), van der Linden & Thijsse (1965), Evans (1969), Einsele & Fiechter (1971), Klug & Markovetz (1971) and Atlas & Bartha (1973).

An achlorophyllous alga, *Prototheca zopfi*, isolated in axenic culture by Walker (1975) has also been found able to chemically alter both Louisiana crude oil and a mixture of paraffinic and aromatic hydrocarbons when grown in estuarine salts solution. Degradation of cumene, n-dodecane and napthalene present in the mixture was almost complete after 14 days.

Criteria for the microbial use of hydrocarbons given by ZoBell (1946) included the disappearance or modification of the hydrocarbon, the production of carbon dioxide, acid formation, multiplication of micro-organisms

or the consumption of oxygen in media consisting of mineral salts solution enriched with hydrocarbons at the sole source of energy.

Many experiments investigating chemical alteration of hydrocarbons by micro-organisms have been directed towards an understanding of metabolic pathways. Many such experiments,especially the earlier ones,have taken one of two forms: growth of an organism at the expense of a hydrocarbon substrate followed by analysis of the growth culture fluid, the cells or both; and the use of 'resting cells' plus substrate followed by a similar analysis. The analyses were concerned with finding oxidative products which reflected the carbon number of the substrate used, or which, on the basis of known chemical reactions could have arisen from the degradation of such intermediates.

One difficulty with this approach is to determine which of the accumulated compounds represents the major oxidative pathway. Products may be directly derived from the substrate but their subsequent accumulation may result from the fact that they are either poorly metabolized or not metabolized at all. Conversely,many oxidation products may be altered soon after formation and thus do not appear in detectable amounts, (Klug & Markovetz 1971).

Alkanes are most commonly degraded via the primary alcohol and aldehyde to the fatty acid of corresponding carbon number following attack on the terminal methyl

group, (Foster 1962). The fatty acids so formed undergo β-oxidation to yield further acids which are successively two carbons shorter. Some micro-organisms are able to attack both ends of the n-paraffins via ω-oxidation with dicarboxylic acids as intermediate products. Others attack n-paraffins by sub-terminal oxidation. A secondary alcohol results from sub-terminal attack and is itself altered to a ketone of corresponding carbon number. Alteration of the ketone leads to the formation and subsequent cleavage of an ester.

Early evidence for the requirement of molecular oxygen in the conversion of alkanes to primary alcohol came from the experiments of Hansen and Kallio (1957). These experiments were based on the principle that denitrifying bacteria are able to use the substrate for oxygen where the sole function of oxygen is to serve as a terminal proton and electron acceptor. In these experiments the bacterium, *Pseudomonas stutzeri* was tested for its ability to oxidize dodecane and various homologues in the presence of nitrate and secondly, oxygen as an oxidant. In the presence of oxygen all substrates, n-dodecane, n-dodecene-1, n-dodecanol, n-dodecanal and n-dodecanoic acid were all oxidized rapidly but in the presence of nitrate only the non-hydrocarbon substrates were oxidized. This was considered evidence that oxygen plays a role in the oxidation of dodecane and dodecene-1 other than one of terminal acceptor and one which is not essential for

the oxidation of the C_{12} alcohol, aldehyde and acid. The work of Leadbetter and Foster (1959 and 1960) provided evidence to further demonstrate the participation of oxygen in reactions other than those of terminal proton and electron acceptor.

Bacterial cells grown on hydrocarbons in the presence of $^{18}O_2$ were found to have an increased cellular oxygen concentration compared to cells synthesized from methanol or glucose. Baptist & Coon (1959) and Gholson & Coon (1960) later demonstrated cell-free enzymic oxidation of n-octanol-1 C^{14} to n-octanol C^{14} in extracts of *Pseudomonas* species verifying that the homologous alcohol is the first stable product of alkane oxidation. More recently, Imada et al. (1967) demonstrated the direct incorporation of molecular oxygen as $^{18}O_2$ into the hydrocarbon molecule showing that the initial reaction is catalysed by an oxygenase and necessarily depends on oxygen.

Hydroperoxidation-reduction has also been proposed as a mechanism to account for the formation of alcohol as the first stable intermediate in methyl group oxidation. Imelik (1948) suggested that hydroperoxide might be formed during the microbial use of alkanes. Subsequently, Stewart et al. (1959) and Finnerty et al. (1962) showed that 1-alkyl hydroperoxides were oxidized by alkane grown cells and Finnerty et al. (1962) demonstrated that a bacterium which formed long chain esters from alkanes also produced the same esters from

the corresponding 1-alkyl hydroperoxides. Other evidence for the mechanism is provided by McKenna & Kallio (1965) who found that cells and cell extracts grown on n-hexadecane were also able to degrade 1-hexadecyl hydroperoxide.

Dehydrogenation has also been proposed as an initial reaction in n-alkane oxidation (Senez & Azoulay 1961) but conclusive evidence is not yet available. Support for the mechanism is provided by experiments which demonstrated that n-alkanes function as hydrogen donors for reduction of indicators by cell extracts under anaerobic conditions. Senez & Azoulay (1961) showed that pyocyanine or N.A.D. was reduced in the presence of n-heptane. Chouteau et al. (1962) later claimed to have identified the alk-1-ene intermediate using I.R. spectroscopy and Azoulay et al. (1963) proposed that following dehydrogenation of the alkane to the alk-1-ene, oxygenation of the double bond would lead to the formation of 1, 2-epoxide which would be reduced to the primary alcohol. Johnson (1964) & McKenna et al. (1965) pointed out however that the energetics of N.A.D. reduction by an n-alkane dehydrogenase were unfavourable and Klug & Markovetz (1967) demonstrated that an organism which can use an alkane of a particular chain length does not necessarily have the ability to use the corresponding alk-1-ene thereby suggesting that alk-1-enes are not likely to be intermediates of alkane oxidation. Alk-1-ene formation from n-alkanes & N.A.D. dependent alkane

dehydrogenation have also been reported by Wagener (1967) and Iizuha (1968) respectively. Klug & Markovetz (1971) suggested that the observed N.A.D. reduction may instead have been due to traces of impurity or the presence of oxygen in the anaerobic experiments. Iso-alkanes are degraded by pathways analogous to those of n-alkanes.

Degradation of alkenes by bacteria fungi and yeasts has been demonstrated by several workers (Bruyn 1954, Stewart et al. 1960, Markovetz & Kallio 1964, Klug & Markovetz 1967, Krause & Lange 1965, Makula & Finnerty 1968, Finnerty et al. 1962.

Oxidation products of alk-1-enes indicate that oxidative attack may occur at several positions (Klug & Markovetz 1971). Isolation of hexadecane -1, 2- diol from cultures of *Candida lipolytica* growing at the expense of hexadec-1-ene was demonstrated by Bruyn (1954) and provided the first evidence for biological oxidation of an alk-1-ene. These observations were confirmed by the work of Stewart et al. (1960). Other experiments with *C. Lipolytica* indicated incorporation of atomic oxygen by oxidation of the terminal double bond with a 1, 2, epoxide as a probable intermediate in the formation of a 1, 2 diol isolated from the system. (Ishikura & Foster 1961).

The experiments of Makula & Finnerty (1968) with *Micrococcus certificans* suggested two mechanisms for the

oxidation of hexadec-1-ene and octodec-1-ene i.e. oxidation of the terminal methyl group as evidenced by ω-unsaturated acids of substrate chain length and double bond oxidation as indicated by a high percentage of substrate fatty acids one carbon shorter than the substrate. A heptane grown pseudomonad grown at the expense of hept-1-ene led to the accumulation of hept-6-enoic acid, further evidence for the attack on the methyl group, (Thijsse & Van der Linden 1963). Similarly, a *Pseudomononas aeruginosa* culture was able to oxidize tetradec-1-ene to tetradec-13-enoic acid (Markovetz et al. 1967). Huybregtse & van der Linden (1967) considered that oxidation of the methyl group resulting in the formation of the α-unsaturated primary alcohol and acid was the most significant pathway of alk-1-ene degradation. Sub-terminal attack leading successively to the formation of a secondary alcohol and monoic acid is also considered a possible mode of attack. (Klug and Markovetz 1968). Watkinson & Somerville (1975) isolated a *Nocardia* species which was able to use the synthetic unsaturated hydrocarbon, butadiene as sole carbon and energy source. Results from experiments with washed cells indicated that degradation was via acetate.

The degradation of cycloparaffins has been reviewed by Atlas & Bartha (1973) and by van der Linden & Thijsse (1965) who concluded that whilst cycloparaffins with aliphatic side chains of sufficient chain length can be degraded, unsubstituted cycloparaffins are generally

recalcitrant to microbial attack. Conflicting results have been reported for the biological degradation of cyclohexane & decalin. Colla & Trecanni (1960) reported that adipic & pimelic acids were produced by the oxidation of decalin by a *Flavobacterium* strain. In contrast to these results, an extensive survey by Pelz & Rehm (1971) failed to discover any micro-organisms able to use decalin.

Growth of micro-organisms at the expense of cyclohexane was first reported by Imelik (1948) and more recently by Fredricks (1966) and Jones & Eddington (1968). Unfortunately a lack of experimental details makes the work of Imelik (1948) difficult to evaluate. The relatively high concentration of substrate (2% v/v) used by Fredricks (1966) was considered by Donoghue et al. (1975) to provide a sufficiently large amount to at least allow the possibility of growth on contaminating material. Jones & Eddington (1968) plated mixed populations from soil extracts onto agar containing basal salts in the presence of cyclohexanone vapour. Under these conditions, interaction of more than one bacterial species would have been possible.

Beam & Perry (1973) found that none of 100 strains of micro-organism tested was able to use unsubstituted cycloparaffins, cyclohexane and cyclopentane as sole sources of carbon and energy and none produced significant growth. However, cyclohexane was degraded in fertile soil as measured by release of ^{14}C-carbon dioxide on addition of ^{14}C-cyclohexane. Hydrocarbon using

organisms isolated from the soil grew rapidly on cycloalkanones and several cultures after growth on propane were found able to oxidize cycloparaffins to the homologous cycloalkanone. The results from these experiments were considered evidence that cycloalkanes in nature may be cometabolized. The work of Beam & Perry (1974) provided further evidence for the cometabolism of cyclohexane in the presence of an alkane.

Complete biodegradation of cyclohexane was achieved by two micro-organisms, an n-alkane oxidizer capable of converting cyclohexane into either cyclohexanol or cyclohexanone during growth on 2 methylbutane and an organism capable of growth at the expense of cyclohexanol or cyclohexanone. Donoghue et al. (1975) also unsuccessfully attempted to isolate an organism capable of growth on pure cyclohexane. They did however isolate strains of *Nocardia* and *Acinetobacter* capable of growth with cyclohexanol. In experiments with cell-free extracts, the alcohol was successively oxidised to the ketone and a lactone via monooxygenase-mediated introduction of a ring oxygen. Hydrolysis of the lactone and subsequent oxidation yielded adipate.

Reports from other workers further suggest that while some hydrocarbons serve solely as growth substrates for particular micro-organisms, others may be simultaneously partially oxidized or cometabolized. A species of *Nocardia* when grown on n-alkanes, oxidized

short and relatively long chain alkyl-substituted cyclic hydrocarbons to cyclic acids. ω-oxidation of the alkyl-substituents was followed by β-oxidation. Residual carboxylic acids with a 2 carbon chain were resistant to further oxidation but cyclic acids with C_1 or C_3 substituents were readily oxidized and used for growth, (Davis & Raymond 1961). In a further study, Raymond et al. (1967) found that *Nocardia* cultures isolated from soil on n-paraffins as a sole carbon source were able to oxidize several methyl substituted mono and dicyclic aromatic hydrocarbons.

Lowery (1962) concluded that co-oxidation probably took place during the growth of individual organisms in complex mixtures of hydrocarbons. In another study, Fredricks (1966) found that several bacteria,including members of the genera *Corynebacterium* and *Pseudomonas*, isolated on media containing n-paraffins of chain length C_{18} - C_{18} readily adapted and grew, as judged by an increase in cell numbers, successfully on branched chain alkanes, shorter chain normal paraffins (C_5 - C_8) and cyclic hydrocarbons. Adaptation in the reverse sense did not apply.

The biological degradation of aromatic hydrocarbons have been reviewed by McKenna & Kallio (1965), van der Linden and Thijsse (1965), Gibson (1968 and 1971), Evans (1969) and Atlas & Bartha (1973). Basic aromatic compounds are converted to catechol which is then converted to cis, cismuconic acid resulting in ring cleavage. The

muconic acid is further transformed successively to oxoadipic acid and succinate & acetyl CoA. The degradation of alkyl benzenes depends on whether the side chain has an even or odd number of carbons. Degradation of the side chain occurs via β-oxidation resulting in the formation of phenylacetic acid and phenylpropionic and/or benzoic acids for even and odd carbon numbers respectively. Benzoic acid can be further transformed to salicylic acid and then to catechol. Phenylpropionic acid can be converted to a catechol and further degraded with ring cleavage. Phenyl acetic acid can be degraded by either of two pathways to give a mixture of acids.

From the results presented by earlier workers it is difficult to generalize about the use of, preference for and rates at which various classes of hydrocarbon are oxidized by single micro-organisms. The reasons for this unpredictability given by ZoBell (1950) were the lack of enzyme specificity of hydrocarbon degrading micro-organisms and the varying degree of dispersion or solubility of hydrocarbons in nutrient media. After discounting differences attributable to these factors, ZoBell (1950) made generalizations based on how rapidly a hydrocarbon could be oxidized and the number of different micro-organisms which could oxidize it. He considered iso or branched chain hydrocarbons to be more susceptible to microbial oxidation than normal or straight chain compounds. He also stated that the longer chain high molecular weight hydrocarbons were more readily attacked

than lower molecular weight compounds and that those with a double bond were degraded more rapidly than their saturated counterparts. Micro-organisms which assimilate aromatic hydrocarbons were claimed by ZoBell (1950) to attack the corresponding aliphatic compound more readily. Fifteen years later in the light of further work some of these generalizations have been refuted; thus, van der Linden et al. (1965) claimed that the hydrocarbons having double bonds were no more susceptible to microbial oxidation than saturated compounds and that straight chained alkanes were more rapidly oxidized than branched compounds. Within the group of n-alkane substrates, C_{12} - C_{16} chains were used for growth (as determined by large increases in numbers) by most strains of *Mycobacteria* investigated by Lukins (1962). The generalizations made by both ZoBell (1950) and van der Linden et al. (1965) do not appear to have been based on good experimental data.

One reason for the lack of definitive information was claimed by Foster(1962a) to be the cost and unavailability of pure hydrocarbons. Criteria for the generalizations made by ZoBell (1950) and van der Linden et al. (1965) were not clearly stated but the nature of the experiments cited suggests that they might have been based on the number of micro-organisms able to use a particular hydrocarbon for growth.

Support for the generalizations of van der Linden et al. (1965) is provided by the nutritional classification study by Soli & Bens (1973). Seawater agar plates were incubated with a mixture of 28 hydrocarbons including

n and isoalkanes, aromatic and alicyclic hydrocarbons. Randomly selected colonies were tested for ability to grow on individual hydrocarbon components. The majority of strains grew on normal alkanes only. Some were able to use compounds from several or all tested hydrocarbon classes. Very few were able to use aromatic or cycloparaffinic compounds in preference to n-alkanes.

Hydrocarbons when added to an ecosystem may selectively enrich or reduce microbial populations. The effects of the hydrocarbons depend upon the chemical composition of the contaminating hydrocarbons and on the species of micro-organism present (Bartha and Atlas 1972). Selective enrichment of those micro-organisms capable of using hydrocarbons and metabolic products and an overall increase in microbial numbers follow the experimental addition of hydrocarbons to a variety of microbial communities.

The presence of hydrocarbons may also reduce microbial populations. Crow et al. (1975) found a decreased number of cellulolytic micro-organisms in salt marsh estuaries exposed to petroleum hydrocarbons. Inhibitory effects of hydrocarbons are related to molecular structure. Liu (1973) found that whilst toluene, ethylbenzene and benzene inhibited microbial growth, aromatic compounds with longer aliphatic side-chains stimulated the metabolic activity of the same micro-organisms. The inhibitory action may be either bacteriostatic or bacteriocidal. Volatile hydrocarbons present in some crude oils have been observed to delay the growth of hydrocarbon degrading micro-organisms until they

evaporate, (Atlas & Bartha 1972, Atlas 1975). A similar observation was made by Kauss et al. (1973) of an algae population. The inhibitory effects of petroleum are often highly dependent upon solubility and concentration. Davis (1967) reported that toluene may stimulate growth of micro-organisms at low concentrations and show bacteriocidal action at high concentrations.

Products resulting from microbial degradation of hydrocarbons may also be toxic. Bell (1971) reported that fatty acids in excess of critical concentrations inhibited the growth of *Candida tropicalis* on hydrocarbon substrates. The critical concentration was a function of solubility which is related to length of the carbon chain. Fatty acids of chain length longer than undecanoic acid were not inhibitory,regardless of concentration due to their very low solubility. The effect of metabolic products on hydrocarbon decomposition has also been studied by Atlas & Bartha (1973). Biodegradation of Swedish crude oil in artificial seawater was more extensive in dialysis than non-dialysis culture suggesting that inhibitory products were formed during growth on petroleum. Fatty acids were produced and were present in the dialyzate. Biodegradation was measured by gas chromatographic analysis of seawater extracts. A similar but less pronounced effect was obtained for a *Flavobacterium* species. Fatty acids present in the dialyzate, when added at various concentrations to cultures of the same bacteria,either inhibited or reduced biodegradation of Swedish crude oil

as measured by decreased oxygen uptake, carbon dioxide evolution, oil biodegradation and protein production.

Some longer chain fatty acids and crude oil were found to have a synergistic toxic effect, which was attributed by Atlas & Bartha (1973) to increased solubility of the fatty acids in crude oil.

Bartha & Atlas (1977) cite a report by Walsh & Mitchell (1973) in which a variety of petroleum components were found to inhibit chemotaxis by a motile marine bacterial isolate. The effect was removed by dilution of the hydrocarbon. Mitchell et al. (1972) considered the inhibition to result from a blockage of the chemoreceptor sites. Affected organisms, although they retained motility, moved randomly and did not respond to available food sources.

When a micro-organism with a broad substrate range is provided with more than one organic substrate, it is likely that it will not attack the substrates simultaneously but instead,in a definite sequence. This phenomenon of metabolic regulation, diauxie, was first described by Monod (1942). van Eyk & Bartels (1968) demonstrated that induction of n-alkane degrading enzymes could be repressed by the addition of glucose, malate and a variety of amino acids. Bartha & Atlas (1977) cited a report by Ward & Brock (1976) in which the addition of glucose to lake water repressed hexadecane use by its microbial community in a diauxic manner. Pirnik et al. (1974) described an experiment in which *Brevibacterium*

erythrogenes was able to use pristane as a sole carbon source only in the absence of n-hexadecane. *B. erthrogenes* degrades n-hexadecane by mono-terminal β-oxidation and pristane by di-terminal oxidation forming dicarboxylic acids which also undergo β-oxidation.

The results suggest that the two pathways are catalysed by different sets of enzymes and that operation of the mono-terminal pathway inhibits operation of the other. This result is consistent with the observation that the n-alkanes of an oil spill disappear before iso-alkanes and other hydrocarbon classes show any substantial change. Bartha & Atlas (1977) believed that an ecological succession of hydrocarbon degrading micro-organisms is also likely to be partly responsible for the sequence of hydrocarbon degradation observed in oil spills. Rapidly growing n-alkane degraders were considered likely to out-compete the more slow growing micro-organisms for oxygen and nutrients, and since they were unlikely to be able to decompose other hydrocarbon fractions (Fredricks 1966), were replaced by other micro-organisms with slower growth. Some experimental evidence for ecological succession has been obtained by Horowitz (1965). Crude oil, depleted by one microbial strain, was used for the enrichment and isolation of another. When four isolated hydrocarbon degraders obtained in this manner were simultaneously inoculated into fresh crude oil, successional changes in population sizes were recorded. Gas chromatography was used to record the pattern of crude oil degradation by

each strain. The two micro-organisms for which high population sizes were recorded initially, were growing at the expense of n-alkanes,whereas those which reached high population sizes after the decline of the alkane degraders decomposed mainly the more recalcitrant and unresolved components of the envelope.

Many workers have investigated the rate of degradation of crude and synthetic petroleum in a mineral salts or saline solution by one or a number of micro-organisms. Based on his own work, ZoBell (1969) stated that he had never found a single species of micro-organism which could noticeably degrade any crude oil but that most crude oils examined were slowly degraded,often almost completely,to carbon dioxide and microbial biomass by mixed cultures consisting of numerous species. He concluded that "most microbial species are highly selective in their ability to attack various constituents of petroleum and products formed therefrom".

In a later study Atlas et al. (1972) showed that species of *Flavobacterium* and *Brevibacterium* were able to degrade 57 and 40 percent respectively of Swedish paraffinic crude oil after 12 d. Degradation was defined as the disappearance of the original hydrocarbons by losses other than volatility. The two species mineralized 42 and 31 percent of the crude oil over 12 d respectively. Composition of the crude oil was not given but it presumably contained mostly paraffinic hydrocarbons.

Biodegradation of hydrocarbons occurs under a wide

range of temperatures. Klug & Markovetz (1967) observed growth of a bacteria on n-tetradecane at 45°C - 70°C and cultures of a thermophilic *Bacillus* species were able to use normal C_{12} - C_{20} alkanes at temperatures ranging from 37°C - 70°C.

Growth and oxygen consumption of micro-organisms has also been reported at temperatures as low as -1°C. The same organisms also grew at 4°C, 8°C and 25°C, (ZoBell & Agosti 1972). Atlas & Bartha (1972) measured mineralization and biodegradation of Swedish crude oil in summer and winter collected nutrient supplemented seawater samples at 5°C, 10°C, 15°C and 20°C. No significant mineralization was observed after 60 days at 5°C for the summer sample but some mineralization was recorded for the winter collected water. A seasonal shift to more psychrophilic forms was suggested by the authors as a possible explanation for the different results. The lag period preceding the onset of carbon dioxide evolution was inversely related to the temperature of incubation and was shown to be caused by inhibitory volatile components present in the oil. Maximum mineralization over 60 d occurred at 15°C and 20°C.

Nitrogen and phosphorus have also been observed to influence the rate at which hydrocarbons are degraded in seawater and mineral salts media, (ZoBell 1969, Miget et al. 1969, Kator et al. 1971, Bridie & Bos 1971, Atlas & Bartha 1972, Reisfeld et al. 1972). Atlas & Bartha (1972) investigated the effects of nitrogen and phosphorus on the

rate of mineralization and biodegradation of Swedish crude oil added 1 percent v/v to seawater. Of the added petroleum only 3 percent was biodegraded and 1 percent mineralized after 18 days of incubation.

A maximum rate of biodegradation was obtained when nitrogen and phosphorus were added together at rates of 140 mg/ℓ and 11 mg/ℓ respectively. Seventy percent of the oil was biodegraded and 42 percent mineralized in 18 days. Incubation temperature was 28°C. Biodegradation was not significantly enhanced when the nutrients were added separately. In another study by Bridie & Bos (1971), maximum biodegradation of Kuwait crude oil in seawater for a given temperature was obtained when nitrogen as ammonium and phosphorus as phosphate were added at rates of 3.2 mg/ℓ and 0.6 mg/ℓ respectively. Natural seawater concentrations were 150 µg/ℓ and 20 µg/ℓ.

Reisfeld et al. (1972) reported that the addition of 10 mg of nitrogen and 1.8 mg of phosphorus per litre of mineral salts medium was optimum for biodegradation of up to 1 g/ℓ of Iranian crude oil by an *Arthrobacter* strain. In 4 days at 32°C approximately 65 percent of the oil was converted to material not extractable by benzene. In an apparatus which provided for continuous addition of nutrients and monitoring of oxygen consumption, 4 µ mol of nitrogen were required for each milligram of Kuwait crude oil oxidized in a seawater medium containing only the natural population of micro-organisms (Gibbs 1975). Approximately 26 percent (based on oxygen uptake) or 44

percent (based on oil recovery) of the oil was degraded and the difference was attributed by Gibbs to the production of soluble organic compounds.

The results of such experiments cannot be extrapolated to the much more complex multiple substrate medium of soil. Activity of hydrocarbon oxidizing micro-organisms has been observed in soil by several workers. ZoBell (1946) observed the gradual disappearance of oil added to soil and marine sediments and found that it persisted in sterilized soils and sediments but gave no experimental details of his observations. In a review by Davis (1967), reference was made to increased bacterial numbers found in soil surrounding gas leaks and attempts made to find petroleum using numbers of methane oxidizing bacteria as in index. ZoBell (1950) pointed out that because hydrocarbons, produced by some plants to the extent of 0.02 percent of the solids (presumably dry matter) content, do not usually accumulate in soil, they must be attacked by micro-organisms. Increases in microbial numbers have also been found where oily wastes had been added to soil (Dotson et al. 1971, Kincannon et al. 1972 and Jensen 1975).

Micro-organisms involved in hydrocarbon decomposition (alteration of the hydrocarbon molecule ranging from its initial oxidation to complete mineralization) have been reviewed by Ellis and Adams (1966). Of the 31 genera of bacteria and fungi listed, all but one were aerobic although large numbers of facultative anaerobes including

Aerobacillus and *Bacillus* spp. were found by Harper (1939) in a soil exposed to natural gas.

In the study by Dotson et al. (1971) the bacterium *Pseudomonas stutzeri* showed the most rapid growth (as determined from plate counts on nutrient agar), after the application to soil of oily waste from oil refining operations including oil-water separations, and from ship ballast water. Waste had been added for about 8 years before sampling and although details of the hydrocarbon composition were not given, the diverse nature of the wastes suggests that a range of hydrocarbons would have been present.

None of the micro-organisms isolated was tested for its ability to use single or multiple hydrocarbon substrates. Kincannon et al. (1972) reported that micro-organisms including species of *Pseudomonas*, *Flavobacterium*, *Nocardia*, *Corynebacterium* and *Arthrobacter* were the most common micro-organisms isolated from oil-contaminated soil as determined by dilution plate counts on nutrient agar. The composition of the three types of oil added, (i) a crude containing a "natural balance of hydrocarbon types" (ii) a high molecular weight oil containing olefinic and aromatic compounds and (iii) waxy raffinate containing highly paraffinic components,did not appear to influence the species of organism present,but the number of bacteria in soil treated with oil decreased in order i, iii, ii. Based on information given in Bergey's Manual of Determinative Bacteriology ed. Breed et al. (1957), few of

the micro-organisms isolated and identified were able to use hydrocarbons.

Jensen (1975) using dilution plates of nutrient agar also found increased numbers of bacteria in samples of soil to which oil had been applied compared to that of the control but no further increase was observed for oil concentrations exceeding 5 percent by weight of soil. Enrichment cultures of the micro-organisms growing on the nutrient agar were made in a mineral salts solution medium with either fuel oil or paraffin oil as a sole carbon source and containing yeast and soil extracts. Pure cultures were isolated by plating on mineral salts medium solidified with 1.5 percent 'Bacto' agar and containing a suspension of droplets of either fuel or paraffin oil. They were tested for ability to grow in a basal mineral salts medium containing 'Bacto' yeast extract and soil extracts. Hydrocarbons added to the medium provided the sole carbon source. The dominant micro-organism present was a species of *Arthrobacter* but lack of experimental details makes his results difficult to interpret. Raymond et al. (1976) investigated the degradation of oil (as measured by weight of soil extracts) in soil to which several mineral oils including car crankcase, truck crankcase, crude, home-heating and residual fuel oil had been added separately at three different localities. Fungi were considered the dominant hydrocarbon degrading flora at only one locality. His conclusions were based on dilution plate counts on both bacterial and fungal agar

media incubated in the presence of hexadacane. These experiments are discussed further on p 138.

The effects of nutrients and simulated cultivation on mineralization of crude oil at low temperatures has also be investigated, (Loynachan 1978). Prudhoe Bay crude oil was added at 0, 3 and 6 percent w/w to a silt loam Arctic soil with an organic matter content of 12.3 percent. Nitrogen as urea, phosphorus as phosphate and sulphur as sulphate were added to the soils at rates of 100, 100 and 50 µg/g respectively to provide treatments N, NP and NPS. The combined effect of N, P, S and micronutrients K, Mg, Fe, Mn, Cu, Zn, B and Mo was also investigated. Cultivation of unoiled and oiled soils was simulated by stirring once after oil addition. In an attempt to promote aeration in the oiled soils birch sawdust (5 percent w/w) was added to the macro-and micro-nutrient amended oiled and control soils.

The treated soils were incubated at 10°C and carbon dioxide evolution monitored periodically for 2h periods. Under the most favourable conditions (nitrogen amended soils) only 18.9 and 11.9 percent of the oil was mineralized after 11 d at the 3 and 6 percent application rates respectively. None of the nutrient treatments further stimulated mineralization rates as judged by total evolved carbon. Cultivation of the soil having 6 percent oil increased mineralization by approximately 10 percent but was ineffective at the lower oil application rate. The addition of sawdust reduced the rate of oil respiration. Aerobic bacterial and actinomycete

populations were higher for the oiled soils but fungi were unaffected. At higher oiling rates the ratio of aerobic to anaerobic bacteria decreased during the course of the experiment. Doubling time for the bacterial population was reduced by 40 percent on the fertilized soil.

When waste lubricating oil is added to soil it is possible that the lead residues present may prove toxic to the soil microflora and fauna. In a recent experiment, Jensen (1977) investigated the effect of lead on the biodegradation of hydrocarbons in soil. Lead as lead nitrate was added at rates of 500 and 5000 µg/g to each of two soils of different organic matter content (2.0 and 9.2 percent) to which fuel oil had been applied at a rate of 2 percent. Treatment and control soils were incubated at 20°C for 4 months. At time zero and after 1 and 4 months, duplicate samples were removed and evolved carbon dioxide measured over periods of about 3 weeks. Mean relative carbon dioxide production from both soil types was considerably reduced in the presence of lead at the higher lead application rate compared to that of the controls. A comparison of the effects of different soil types was not possible due to inconsistencies in the data and a lack of statistical treatment. Bacterial composition of the soil was unaffected by the presence of lead but numbers decreased on both soil types. Fungal species, *Paecilomyces lilacinus* and a *Fusarium* species dominated the lead treated soils. The amount of fungal mycelium increased in the presence of both oil and lead

although fungal counts were unaffected. The dominant fungal species were known to be active decomposers of alkanes and a further experiment was carried out to test the possibility that the microflora may have partially adapted to the presence of lead.

n-hexadecane was added repeatedly to soil samples containing different concentrations of lead and oxygen consumption continuously recorded. The results showed that delayed decomposition of the hydrocarbon in the presence of lead was caused solely by a prolonged lag phase. When this period passed, activity in the presence of 5000 μg Pb/g was the same as that recorded for the control soil. Following a second addition of n-hexadecane the same or possibly slightly stimulated activity resulted. In another experiment, fuel oil instead of n-hexadecane was added to the same soil samples at intervals of 20-25 d. The initial lag phase was almost completely absent after the second addition of oil and increased oxygen consumption was observed. A lag phase, present after the addition of oil to a lead amended soil, (5,000 μg/g) was reduced following a second addition but increased again following a third. It was surmised by the author that while the alkane fraction of the fuel oil was decomposed, degradation of other oil fractions had been inhibited in the presence of a high concentration of lead. The duration of these experiments, up to 4 months, was relatively short and it is conceivable that with time, other organisms would have become adapted to provide the enzymes needed to decompose

the remaining oil fractions.

The effects of lead on soil respiration has also been investigated by Doelman & Haanstra (1979). Lead as lead chloride was added at concentrations varying from 375 to 7500 µg/g to four soil types, two sandy soils of different organic matter content, a clay and a peat soil. Respiration as measured by oxygen uptake of all soils except peat was reduced at the lowest lead concentration. The amount of inhibition increased with increased lead concentration and was greatest for the sandy soils.

To investigate the long term effects of lead, the respiration of lead amended 'low' organic matter (2.8 percent) sandy soil, was measured after 1, 2, 3, 5 and 40 months. The respiration rate of the treated soil after 40 months was still 70 percent of the unamended control. Dehydrogenase activity of the amended and control soils was also measured. Reduced activity was recorded only for the lead amended sandy soils. The severely reduced respiration rate and unaffected dehydrogenase activity recorded for the clay soil suggested that respiration rate is more sensitive to the inhibitory effects of heavy metals.

There is also evidence that lead, applied to soil in sufficient concentration as an inorganic salt, may migrate both vertically and horizontally. Stevenson et al. (1979) applied lead as lead acetate to a silty clay loam at rates of up to 3200 kg/ha. The lead was incorporated into the top 15-20 cm of soil by disking. Increased lead concentrations in the 15-30 cm soil interval were obtained

for 800, 1600 and 3200 kg/ha lead application rates. Contamination below 30 cm was recorded only for 3200 kg/ha treated soil. The lead concentration for the interval 75-90 cm was approximately 18 percent higher than that of the unamended control. Mechanisms postulated by the authors to explain the vertical migration of lead included the leaching of soluble complexes with natural chelates formed during the decay of organic residues, transfer of soil to lower depths by earthworms and other macrofauna, plant uptake and downward translocation in plant roots and physical transfer of soil in fissures and cracks formed by soil dessication during dry periods. Horizontal migration of lead in the surface layer was attributed to wind blown dust.

Other studies made suggest that some soil micro-organisms are insensitive to high concentrations of lead. Tornabene et al. (1972) found that the bacterium *Micrococcus luteus* and a species of nitrogen fixing *Azotobacter* were able to immobilize respectively, 4.9 and 3.1×10^2 mg of lead/g of whole cells on a dry weight basis. Lead added to cell cultures as bromide, iodide and bromo-chloride in concentrations approaching solubility limits had no detectable effect on growth rate and cell viability. Almost all the lead was immobilized in the cell wall and cytoplasmic membrane fractions but details as to where and how it occurred were not given. It is conceivable therefore that there could be hydrocarbon oxidizers in the soil which also have a capacity to tolerate large concentrations of

lead.

The fauna of many soils may also be insensitive to high concentrations of lead. Williamson et al. (1972) reported concentrations of 700 ppm of Pb on a weight basis in the bodies of woodlice including *Philoscia muscorum* caught near a roadside but only 30 ppm in their mammalian predators suggesting at least that the metal is not concentrated in some food chains. The form in which the lead occurred was not studied. In a further study the same authors,(Williamson et al. 1973) found no effects on numbers of the major groups of invertebrate fauna from spoil of a disused lead mine the soil of which contained lead in concentrations up to 9,000 ppm.

Several workers have observed a range of short term changes to the physcial and chemical properties and conditions of soil contaminated with hydrocarbons. Weak soil structures are broken down where oil is added and deflocculation takes place in such soils which although hydrophobic tend to remain wet once wetted (Ellis et al. (1961). The higher field capacity and lower wilting point relating to an observed increase in porosity and decrease in density of gassed soils may mean that more water is available for use by plants.

The colour of soils contaminated by gas or oil also changes, lighter soils becoming darker (Op. cit.). Ellis et al. (1961) also observed changes in pH of soils contaminated with natural gas: for acidic soils an increase and alkaline soils a decrease. Products of gas

saturation may therefore tend to buffer the soil towards neutrality. Jensen (1975) also found the pH to be lowered where oil hydrocarbons (as oil sludge) were added to soil. Other changes included large increases in organic matter, total carbon and nitrogen compared to nearby uncontaminated soil. Harper (1939) claimed the average nitrogen and organic matter content of soils exposed to natural gas from leaking pipelines to be 2.5 - 3.0 times that of nearby unaffected control soils but lack of soil sampling details suggests his results may be suspect. Ammonia was detected by Harper (1939) in the natural gas at only very low concentrations (0.01 ppm) which suggested that the increased nitrogen content of the 'gassed' soils was not due to the absorption by the soil of ammonia from the natural gas. Nitrogen fixation by the obligately anaerobic *Clostridium* spp. present in the gassed soils was considered by Harper (1939) to be a possible explanation for the observation. Similar trends were observed by Plice (1948). Soil around a natural gas leak contained 12.47 percent organic matter and 0.81 percent nitrogen. The unaffected soil 60 cm distant contained 0.52 percent organic matter and 0.03 percent nitrogen. Only 0.27 percent of the soil (presumably by weight) could be extracted by tetrachlorethane, a solvent which Plice (1948) claimed was suitable for most hydrocarbons.

Similar trends were obtained by Plice (1948) for both sandy loam and clay soil mixed with oil. An analysis of 'paraffin dirt', a natural deposit, by Davis (1952)

showed that it contained 1.2 percent Kjeldahl-nitrogen, compared to 0.1 percent organic nitrogen for adjacent control soil. Plice (1948) claimed that the oil and natural gas used in his experiments were nitrogen free but no analyses of these materials were given. It is therefore difficult to accept his conclusion that fixation of nitrogen (presumably of atmospheric origin) had occurred in the amended soils. Crude oil and natural gas contain an average of 0.1-0.5 and 1-15 percent nitrogen respectively (Levorsen 1967). It occurs as nitrogen gas in natural gas but little is known about its nature in undistilled crude oil (Levorsen 1967).

Analysis by Numanov et al. (1974) of 66 petroleum samples from different parts of Russia showed total nitrogen contents varying from 0.15 to 0.17 percent of which 21-58 percent was tertiary amino nitrogen. The observed increase in the nitrogen content of the oiled soils may therefore have been due to the absorption of nitrogen containing oil by the soil.

Changes have also been observed in the nutrient status of hydrocarbon contaminated soils. Ellis et al. (1961) suggested that the greater amount of 'plant available' phosphorus present in most gas saturated soils compared to controls may be due to a more favourable pH and that some phosphorus would be brought into solution by reducing conditions that make iron phosphates more soluble. Adams et al. (1960) also observed an increase in the manganous and ferrous ions in contaminated soils.

Values of 60 and 120 ppm for exchangeable manganous and ferrous ions respectively were obtained for gas saturated soils. Nitrate formation was reduced by the addition of oil to soil and ceased at a concentration of 1 percent oil by weight.

When this thesis was begun, there were very few reports of microbial decomposition of waste lubricating oil. Ludzack et al. (1956a) followed the decomposition of waste motor oil over 28 d by measuring the evolved carbon dioxide in an air recirculated system. Oil was added at a concentration of approximately 25 mg/ℓ to 4 ℓ bottles containing water and sewage as an inoculum. Oil concentration was measured at the end of each week by infrared analysis (Ludzack et al. 1956 b) and sufficient oil added to raise the concentration to approximately 25 mg/ℓ. Air was introduced at a rate that would assure at least 60 percent oxygen saturation. The amount of sewage inoculum added was 10 percent by volume of the bottle and carbon dioxide corrections were based on biological oxygen demand of a similar amount of inoculum. Complete oxidation (mineralization) of 244 mg of oil (based on 3.12 mg of CO_2/mg hydrocarbon) was obtained during the 4 week period at 25^{o}C (or 75.3 percent of the total added). Maximum carbon dioxide production was recorded on day 10. The 966 mg of carbon dioxide collected over 28 d was 206 mg in excess of that calculated from oil disappearance. B.O.D. of the sewage seed was 220 mg. Good agreement between anticipated and observed carbon dioxide production

indicated that carbon dioxide was the principal end product of oil oxidation. Intermediates formed included acids and esters. Oil oxidation at 4°C was not measurable as determined by emulsion characteristics, infrared curves and oil concentration after 60 d.

No reports have been found in the literature of the extent to and rate at which various components of waste lubricating oil are altered biochemically in soil but these factors will presumably depend in part on what enzymes are present in the soil. Conceivably the first time a hydrocarbon is added to soil it may take some time to decompose. This delay in decomposition may be due to the need for the microflora present to provide the enzymes necessary to decompose hydrocarbons. Raymond et al. (1976) in a recent field experiment applied various oils including waste car and truck crankcase oil separately to soil at a rate of 30 t/ha and on the basis of soil extracts claimed an average rate of degradation or biodegradation of 6t/ha/month. The terms degradation and biodegradation were not defined and appeared to have been used synonymously by the authors.

Knowledge of hydrocarbon assimilation by soil micro-organisms although small has been applied in the U.S.A. where land disposal has been used to treat sludges and emulsions from oil and water separators and oil laden ship ballast waters.

Dotson et al. (1971) applied oily waste to 0.5-1.0 ha plots of clay rich poorly drained soil of neutral or

calcareous pH. The plots were surrounded by 0.5 m borders to prevent loss of oil from the plots. Excess water and oil was drained off the sloping land into a trap from which any accumulated oil was pumped back onto the treated plots. The time required to "decompose" the hydrocarbons applied at 300 t/ha varied from 3-9 months. "Decomposition" was not defined but the statement by the authors that "...the soil returns to a brown friable condition indicating that biological oxidation has progressed far enough for oil to be added again" suggests that it was based on a visual assessment of the soil structure. No evidence was given for biological oxidation of the oil.

In preliminary pot trials, Dotson et al. (1971) found that oily sludge mixed with soil and remixed at intervals "decomposed" at rates varying from 80-960 g/dm^3/ month (120-1440 t/ha/month*). As much as 23 percent by weight added to soil was claimed to "decompose" at a rapid rate but no details were given.

Kincannon et al. (1972) investigated the disappearance of oil from 150 m^2 cultivated plots and claimed that decomposition rates of oil averaged about 8 g oil/dm^3 soil/ month (or 12 t/ha/month*) and 16 g oil/dm^3 soil/month (or 24 t/ha/month*) in those plots that received nitrogen, phosphorus and potassium fertilizer.

*Based on data of Kincannon et al. (1972) and assumes that 1.0 ha of soil to a depth of 15 cm weighs 2.5 x 10^6 kg. (Plots cultivated to a depth of 12 cm).

Sludge (20 percent hydrocarbon or about 110 t/ha) has also been dumped on grassland, partially dried and then disked once into clay soil, pH 4.4-4.8 (Dotson et al. 1971). A single application of 2.04 t of ground limestone, 56 t/ha nitrogen and 67 kg/ha phosphorus was made to the disposal site but time of application was not given. By the time the sludge was dry enough to work into the soil the grass had started to grow and had established normal cover within a year. A similar project (Anon 1972b) has been carried out in California to dispose of 1.6×10^5 m^3 of drilling muds and other waste accumulated over 40 years from drilling operations. Waste material was spread on land and disked into the soil. Disking was repeated every 3 d when more waste was added. No details of oil application rates or what happened to the waste after its application to the soil were given.

Results of an investigation by Schwendinger (1968) suggested that if crude oil is added to soil at concentrations greater than 1 percent by weight both germination and crop yield are reduced. In a seed germination experiment, Schwendinger injected crude oil into 100 ml beakers of soil at rates 1-2 and 5 percent (w/w) and claimed that germination of oat seeds was reduced by 75 percent when 5 percent oil was added but that there was no reduction at the lower level. Similarly shoot dry matter yield of ryegrass grown in pots of loamy sand "fertilized adequately" and injected with crude oil at rates 1:60 and 1:30 oil:soil v/w and depths 2, 7 and

14 cm was reduced by up to 30 percent. Treatments were not duplicated and a lack of experimental detail makes his results difficult to interpret. Murphy (1929) found that wheat stands were reduced to 23 percent of the control when oil had been added to the soil surface at a rate of 28×10^3 ℓ/ha (assuming Sp.G. = 1, 28 t/ha). Oil at a similar application rate but mixed with the soil prevented seed germination.

Reduction in shoot dry matter yield of plants grown on oily soil may diminish with time. Plice (1948) added crude oil to soil (1 percent w/w) and found no reduction in yields of wheat, barley and rye grown 6 months later while the yield of crops grown after 3 years increased by 20 percent. He attributed this stimulation partly to an increase in nitrogen content of the contaminated soil. He also claimed that the reduction in plant yield resulted mainly from the physical conditions of the contaminated soil rather than toxins but gave no experimental evidence to support these claims.

In the pot experiment, Johansson (1962) added 3 percent oil to a clay soil (6.0 percent humus) and a loamy sand (2.6 percent humus) and measured the straw and grain yields of wheat grown on the oiled soils for 10 years after oil application. One crop was grown each year and more oil applied at the same rate 12 months after the first application. Reduced straw and grain yields compared to those of the controls were obtained for the first 4 years on both soils but normal yields were obtained after the

fifth year for plants grown on the clay soil. Eleven years after the first oil application the straw and grain yields obtained on the loamy sand were the same as those of the control. He concluded that a high clay and humus content increased the "buffer capacity" towards oil contamination.

During the combustion of petrol a variety of lead compounds may be present on the internal surface of internal combustion engines depending on engine operating conditions. These include halides, oxy-halides, oxides, oxy-sulphates and sulphates. Any or all of these may occur in waste lubricating oil (Shell Oil New Zealand Ltd. pers. comm. 1973). Conceivably there could be an accumulation of lead residues in soil which has been used for the disposal of such waste. While there is no literature concerning the uptake of oil derived lead residues by plants from soil there are several reports, (Broyer et al. 1972, Page et al. 1971, Maclean et al. 1969) that have suggested that if lead is applied to soil or solution culture as an inorganic salt e.g. nitrate, lead accumulated in the roots. There was however very little transport to the shoot system. Jones et al. (1973) showed for lead applied as nitrate to ryegrass *Lolium perenne L.*, variety S23 growing in 1 *l* of solution containing 281-9969 µg/Pb/3 plants that transport to the shoots over 28 d did not exceed 28.9 percent of total uptake. In a field experiment Baumhardt et al. (1972) added lead as acetate to soil at levels 0-3200 kg/ha and found that

emergence, plant height and grain yield of corn were unaffected. Similarly the lead content of the grain was not significantly greater than that of controls.

The amount of lead taken by plants depends on several factors. Keaton (1937) grew barley *Hordeum vulgare* on soil to which lead had been added as nitrate and carbonate and found the total lead uptake to be 31 percent greater for soil to which it had been added as nitrate. The addition of phosphorus to soils and plant culture solutions was considered by Rolfe (1973), Miller et al. (1970) and Maclean et al. (1969) to repress the amount of the metal taken up by plants. Miller et al. attributed repression of $PO_4^{=}$ uptake to selective uptake under conditions of $PO_4^{=}$ sufficiently rather than to precipitation of lead phosphate in solution. Cox et al. (1972) found repression of shoot growth by lead for several plant species when lime was added to soil. They suggested that the effect was related to availability because lime had no effect on plant yield. Similarly a reduced amount of lead in the above ground portion of oats *Avena sativa L.* and alfalfa *Medicago sativa L.* grown on soils with a higher organic matter content was found by Maclean et al. (1969).

Season also appeared to influence lead uptake by plants. According to Mitchell et al. (1966), the normal lead content of rotational mixed pasture herbage grown on a variety of soils increased from 0.3-1.5 ppm in dry matter during the period of active growth to 10 ppm and reached 30-40 ppm in late winter. The increased lead content of

the above ground portion of the dormant plant was suggested to be due to movement from the root rather than active lead uptake.

Little is known about lead toxicity in animals. Hammond et al. (1964) attributed the death of cattle and horses to feed contaminated with atmospheric lead from internal combustion engines and lead smelters and concluded that grazing crops containing 120-150 ppm lead would produce death in the animals.

The survey of the literature indicates the need for further work especially that concerning oily waste disposal. No standard criteria upon which the relative recalcitrance of hydrocarbon molecules can be determined have been established. Extrapolation from the results of such experiments in a liquid medium of oily soil with more than one microbial substrate and a complex microflora is made more difficult by variation in the availability of hydrocarbons to micro-organisms arising from the adsorption of hydrocarbons by inorganic soil components. A measure of persistence due to resistance or unavailability to biochemical/chemical alteration of hydrocarbons is more appropriate to the soil medium.

In many investigations especially those concerning the disposal of oily wastes in soil, terminology concerning the biochemical modification of hydrocarbons has been used but not defined. Results obtained from many such experiments are difficult to interpret because of imprecise methods and are discussed in Chapter VII.

Where land is used to dispose of waste lubricating oil, the amount of land required is an important consideration. Thus persistence in economic terms must relate to the amount of oil which can be applied/unit area/ unit time,a suitable measure of which might be its rate of disappearance from soil without loss to sub-soil, surface runoff water or ground water, and without deleterious modification of the biological properties of the soil.

The following investigations attempted to ascertain the factors and processes affecting the disappearance of waste lubricating oil from soil and to determine the effects of oily residues on the biological properties of the soil with the object of evaluating the feasibility of land disposal.

CHAPTER II

THE LOSS OF WASTE LUBRICATING OIL FROM TIMARU SILT LOAM UNDER FIELD CONDITIONS

I. MEASUREMENT OF OIL LOSSES FROM SOIL

(1) Review of Methods Available

(a) Labelling Selected Hydrocarbons. One or more C_{14} labelled hydrocarbons normally occurring in lubricating oil could be mixed with the waste prior to its application to the soil. The activity of samples removed from the field could be measured and the rate of loss computed.

(b) Respiration. The measurement of oxygen uptake or carbon dioxide evolution by soil is a technique often used to measure microbial activity (Ludzack et al. 1956a Mokhtar 1973). Respiration measurements including CO_2 released from C_{14} labelled hydrocarbons obtained for field samples would provide information on the relative levels of activity, both chemical and microbial.

(C) Gas chromatography. Combined gas chromatography and gravimetry of soil extracts has been used to study oil disappearance from soil by Kincannon et al. (1972) who chromatographed weighed extracts of oil that had been laden with oil and measured changes in the relative amounts of compounds of different boiling points.

(d) Gravimetric Analysis. The weighing of extracts of oily soil was used by Kincannon et al. (1972) and Raymond et al. (1976) to follow losses of oily wastes from soil under field conditions. In the former study, samples of oily soil were Soxhlet extracted and in that of Raymond, each 50 g sample was extracted in a 400 ml beaker containing 100 ml of boiling benzene. After mixing soil and solvent with a teflon coated stirring bar, the mixture was permitted to settle and the benzene decanted off. The procedure was repeated three times with 50 ml aliquots of benzene.

(2) Factors Affecting Choice of Method

Before a suitable method for measuring oil loss from soil could be selected it was necessary to consider ways in which oil might be lost from soil, composition of the soil and the number of measurements required to provide a statistically reliable estimate of oil concentration in the soil.

(a) Possible Loss of Oil from Soil. Oil losses from soil may occur through direct evaporation of the hydrocarbons or direct volatolization of oxidation products resulting from the microbial transformation and chemical oxidation of hydrocarbons, Plice (1948), Davis (1967). Hydrocarbon loss is said to be promoted by alternate wetting and drying of soils and by high temperatures (Plice 1948) while chemical oxidation is a function of aeration, temperature, surface area and the presence of free radicals, (Davis 1967). Photo-oxidation of hydrocarbons has been demonstrated in vitro by

Burwood et al. (1974) for solubilized fractions of crude oils in sea water and could occur where waste lubricating oil is mixed with the top few cm of soil. The extent of surface runoff and sub-soil leaching is dependent on the amount and intensity of rainfall, physical and chemical properties of the soil, water solubility of the hydrocarbons, the formation of emulsions and the presence of detergents. Oil may be irreversably adsorbed, as judged by its failure to be removed by Soxhlet extraction with a solvent in which it it soluble, onto the surface of soil particles (Loran et al. 1973) and there is evidence that oil may be degraded or oxidized by micro-organisms,(Ludzack et al. 1956a), Atlas et al. 1972). Such evidence is mainly from studies in which micro-organisms isolated from oil contaminated soil have been shown able to use hydrocarbons as sole carbon source or oxidize them whilst growing at the expense of others present.

(b) Oil Composition. Lubricant wastes consist mainly of straight chain aliphatic hydrocarbons of general formula C_nH_{2n} with a molecular weight range between 400 and 750 (Shell Oil New Zealand Ltd. pers. comm. 1973a). In addition there are minor amounts of organic additives including (i) detergents (oil-soluble soaps comprising a metal e.g. aluminium, magnesium, zinc, barium, calcium, an anionic component e.g. carboxylate, alcoholate and phenate, sulphonate and salicylate and oleophilic component, usually a straight- or branched-chain alkyl groups of appropriate molecular

weight e.g. barium phenate), (ii) dispersants (polypolar polymers and polyalkenyl succinimides), (iii) anti-oxidants (phenols e.g. α-naphol and metal dithio-phosphates e.g. zinc dithiophosphates) (iv) viscosity index improvers, high molecular weight polymers e.g. polymethacrylates and polyolefins - molecular weight range 5,000 - 20,000), (v) pour-point depressants e.g. polyalkylmethacrylates, napthalenes, alkylated wax phenols, alkali- metal sulphonates (vi) extreme pressure agents e.g. organic sulphides or disulphides and (vii) rust inhibitors e.g. high molecular weight carboxylic, sulphonic or phosphoric acids, (Ford 1968, Peet 1969). Some of these additives e.g. anti-oxidants function by chemical reaction and will therefore occur in modified form in waste lubricating oil. The additives together total only 2-4 percent by weight of the waste lubricating oil. Benz-3,4-pyrene, considered to be a carcinogenic hydrocarbon by Heidelberger (1964), occurs in used lubricating oil at concentrations of 0-10 ppm by weight, Thony et al. (1975). Minor amounts of kerosene used for cleaning engine parts may also be present in the oil.

(c) Number of Samples Required. When waste lubricating oil is first mixed with soil on a field scale its concentration over a plot may, depending on the method of cultivation used, vary due to imperfect mixing. A large number of samples may therefore be necessary to obtain a statistically reliable estimate of the oil concentration for a field plot.

(3) Choice of Method

Of the methods outlined for the measurement of the oil loss from oily soil, all have disadvantages. Labelling selected hydrocarbons would enable a large number of samples to be processed in a short time but it would be necessary to assume that the labelled hydrocarbons behaved in a similar manner to that of the hydrocarbons occurring in the waste and because micro-organisms use some hydrocarbons preferentially and in different ways (ZoBell 1950, van der Linden et al. 1965) this assumption cannot readily be made. The results obtained by this method could not easily be expressed in a form appropriate to engineers because some hydrocarbons, while not degraded (loss of carbon atoms from the molecule) may have been oxidized to form intermediate products.

While it would be possible to measure respiration rates in the field, the number of samples required for a large field experiment would necessitate the use of sophisticated analytical equipment which was unavailable to the project. Furthermore, results could not be easily interpreted in terms of oil loss.

Quantitative information on the composition of oil which has been mixed with soil, subsequently extracted and analysed by gas chromatography depends on complete recovery of oil present in a representative number of samples. No information relating to recovery rates was given by Kincannon et al. (1972) and it is therefore difficult to interpret his gas chromatography studies. Each analysis may take several

hours and the time required to obtain statistical reliability for a large field experiment may be prohibitive.

Gravimetric analysis of soil extracts was selected for the present study because it has several advantages over the other methods outlined. It is a simple and rapid method which, providing the recovery rate for oil from soil is known, may be used to measure most oil losses from soil and is suitable for the large number of soil samples required to give a reliable estimation of oil concentration in a field plot (see p 74). With repeated mixing of oil and soil in field plots it is conceivable that the oil would become more evenly distributed through the soil volume. An increased proportion of adsorbed oil may result thereby affecting the percentage oil recovered and complicate interpretation of results obtained from a time course experiment. Waste oil hydrocarbons (such as those derived from internal combustion engines) recovered from the extract would include only those which have been insufficiently altered to render them insoluble in the extracting solvent. Others could be mineralized (degraded to CO_2 and H_2O) by soil micro-organisms or be partially altered. Oil loss measured by gravimetric analysis is not therefore synonymous with complete mineralization of the oil. Finally loss attributable to combined chemical and microbial activity would be circumstantial.

(4) Preliminary Experiments

Both Kincannon et al. (1972) and Raymond et al. (1976) used gravimetric analysis to follow the disappearance of oil from soil but neither study gives any information concerning oil recovery rates for oil added to soil.

The following experiments were performed to find a suitable gravimetric method for recovery of waste lubricating oil from soil.

(a) To Determine the Proportion of Hexane Insoluble Matter in Waste Lubricating Oil. Experiments with various solvents suggested 'Pegasol 1516', an aliphatic solvent (Mobil Oil New Zealand Ltd) to be a suitable solvent for waste lubricating oil mainly because of its relatively low boiling range 66°C-70°C which would reduce the risk of extract loss when the solvent was removed. Waste lubricating oils contain in addition to hydrocarbons, suspended carbon and fragments of bearing metal and additives as outlined on p 48 and it was necessary to determine the proportion of solvent insoluble matter in the waste before considering the extraction of oil from soil. One g of waste lubricating oil left undisturbed for several weeks and 50 ml of distilled 'Pegasol 1516' solvent were added to a 100 ml Ehrlenmeyer flask which was then shaken vigorously for 2 min. To avoid significant contamination of the oil after solvent removal, the 'Pegasol 1516' was distilled to obtain the fraction boiling between 66°C and 68°C. This fraction shall be referred to as 'hexane' in the remaining text. The flask

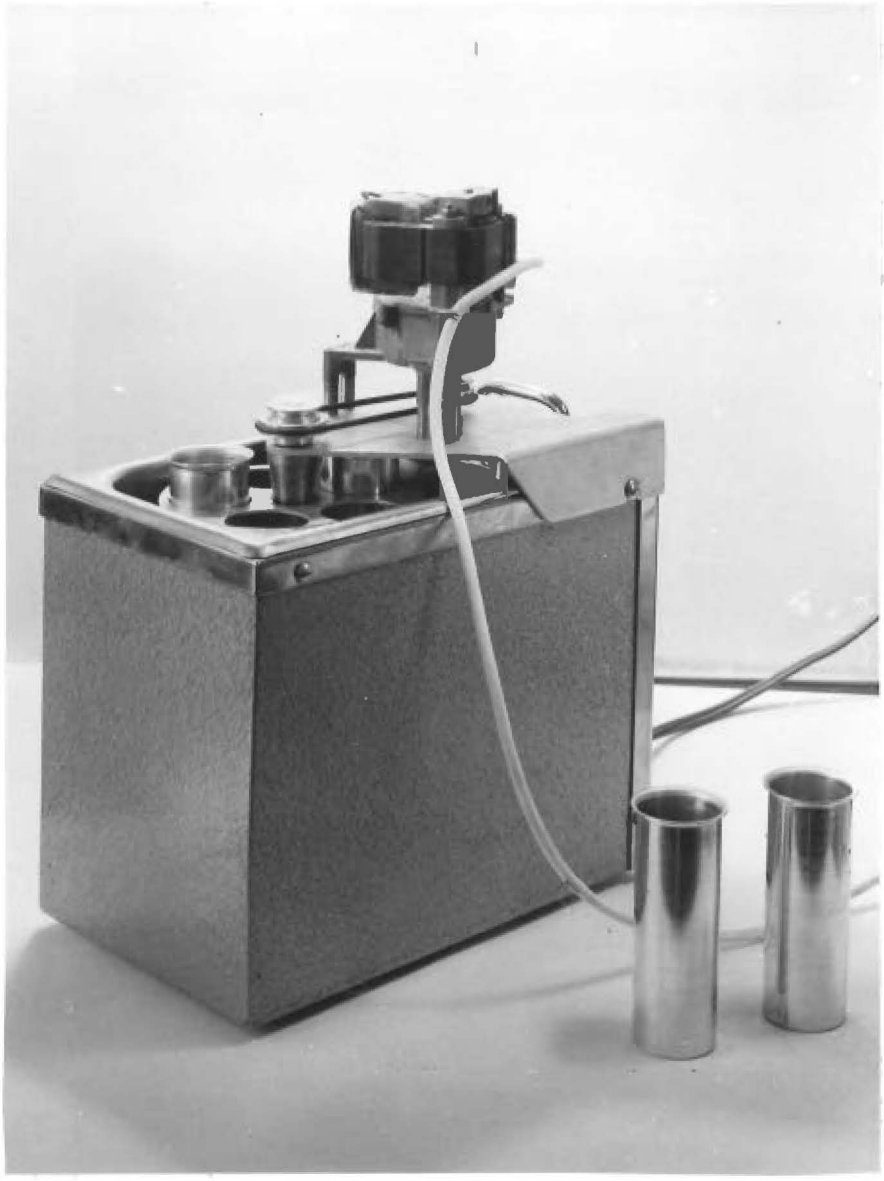
contents were vacuum filtered through a 0.45μ cellulose ester millipore filter, (chemically non-reactive with hexane) and the flask rinsed with a further 50 ml of solvent. The rinsing was also filtered and the filtrate transferred to a 100 ml flask. Solvent was removed by evaporation under reduced pressure using a Buchii rotary evaporator (water bath temperature 28°C). A silica gel filtered air-stream was used to remove any residual solvent from the flask. Silica gel was used to remove in addition to water, any oil from the compressed air-line. The resulting residue was weighed to 0.01 g ± 0.005. The procedure which was duplicated twice gave 100 percent recovery within weighing error for all samples. Suspended matter in the waste lubricating oil was responsible for its dark colour and because most of it was not removed by filtration accounted for less than 0.005 g. Residue weight obtained for pure hexane controls was less than 0.005 g.

(b) To Compare the Efficiency of Three Methods for Extracting Waste Lubricating Oil from Soil; Insonation, End over End Shaking and Standing Extraction.

Method (1)

A motor driven rotating assembly of 5 aluminium beakers constructed to fit into an ultrasonic tank was used for insonation (Plate 1). Speed of rotation was 12 rev/min and 400 ml of 10 percent 'Teepol' (Peterson Chemicals Ltd. 122 Bamford Street, Woolston Christchurch, 2, New Zealand)

Plate 1: Insonation equipment



detergent solution was used to aid energy transmission. Attempts to monitor the wave pattern of the bath when at its maximum energy output gave a complex pattern suggesting periodic dependence of cavitation intensity on height of bath solution as shown by Weissler (1961) to be unimportant. The assembly rotated a constant rate and beakers of oil and solvent should therefore have received equal amounts of energy thereby avoiding the problem of reproducibility.

Method (2)

Four cork-sealed 15 x 2.5 cm test-tubes attached vertically by clips to a motor driven rotating disk were used for end over end shaking. Speed of rotation was 5 rev/min.

Method (3)

Aluminium beakers as used for sonification were employed for standing extraction in which solvent was added to soil with no subsequent dispersion.

Approximately 5 kg of soil randomly sampled at a Timaru site, (grid reference Lands and Survey Department N.Z.M.Z.1 S111/784488) later used for the field experiment, was collected to compare the extraction methods. The soil, an impermeable grey brown clayey silt overlain by a 7-8 cm dark greyish brown friable silt loam 'A' horizon having a weakly developed nutty structure and an organic matter content of approximately 2.5 percent, had previously been cultivated and had received lime and super-phosphate (11.5 t/ha and 68 t/ha respectively, see p.67).

Four hundred g of air-dried soil, ground in a mortar with a pestle to pass a 4 mm sieve and containing 6 percent water by weight was weighed into each of two 1 ℓ beakers and waste lubricating oil mixed thoroughly with the soil to provide oil concentrations of 10:1 soil: oil w/w (beaker A) and 50:1 (beaker B). The oil/soil mixtures were stored at 4°C for 48 h, the time required to complete the experiment. Thirty ml of hexane was added to each of five 5 g ± 0.005 samples of soil from beaker A and the soil/hexane mixtures sonified for 1 min. The extract/solvent phase from each soil sample was decanted and filtered through a pre-weighed fluted Whatman's no. 1 filter paper. The filtrate was collected in a 100 ml centrifuge tube. After a second sonification with a further 30 ml of solvent both the extract/solvent phase and soil were quantitatively transferred to the same paper filter. Remaining residue was washed into the latter with an additional 20 ml of solvent. After centrifuging at 3,500 rev/min for 10 min the extract/solvent phase from each filtrate was poured into a pre-weighed pear-shaped 100 ml flask. Solvent was removed by vacuum under reduced pressure (water bath temperature 28°C). Residual hexane was driven from the extract by an air stream dried with silica gel (1.5 ℓ/min) for 20 min. Flasks were reweighed and the extract weights recorded to 0.01g ± 0.005. This procedure was repeated for soil from beaker B and again for both soils with a 10 min extraction time. Using the same procedure further soil samples were

extracted by end-over-end shaking and standing extraction.

Results

Results are shown in Table 1 . Significant F values were obtained for both low and high soil oil concentration. Less oil was recovered by end-over-end shaking for both extraction times at the higher oil concentration and for the 10 min extraction time at the lower oil concentration compared to the amounts removed by the other two methods which were approximately equal. This pattern of results would be expected where strongly disaggregated soil had reduced the filtration rate. The loss of a greater amount of solvent from the filters during filtration would leave more viscous oil adhered to the filter and hence give a lower recovery rate. A visual comparison of filters indicated this to be the case. Standing extraction was employed for subsequent experiments because it was rapid and convenient and is used as described above with a 1 min extraction time/solvent fraction.

(c) To Determine the Effect of Soil Type on the Recovery Rate of Waste Lubricating Oil Added to Timaru and Templeton Soils

A further experiment was carried out to determine the effect of soil type on the recovery rate of waste lubricating oil.

Timaru soil (p 65) which had been cultivated (p 67) in preparation for a field experiment was air-dried and ground

and standing extraction with hexane. Means of 5 replicates.

Extraction method	Ratio oil: soil	Oil applied g/5g soil	Oil yield g/5g soil 2 x 1 min extractions	Percent oil recovered	Oil yield g/5g soil 2 x 10 min extractions	Percent oil recovered	
1 Sonification	1:10	0.45	0.37	82.2	0.38	84.4	F = 15.77** S.E. = ± 0.01039 L.S.D. (5%) = 0.05
2 End over end shaking	1:10	0.45	0.32	71.1	0.28	62.2	
3 Standing extraction	1:10	0.45	0.35	77.8	-	-	
1 Sonification	1:50	0.10	0.09	90.0	0.09	90.0	F = 3.08* S.E. = ± 0.00224 L.S.D. (5%) = 0.01
2 End over end shaking	1:50	0.10	0.09	90.0	0.08	80.0	
3 Standing extraction	1:50	0.10	0.08	80.0	-	-	
1 Sonification	Unoiled control	0.00	N.D.	0.0	N.D.	0.0	
2 End over end shaking	Unoiled Control	0.00	N.D.	0.0	N.D.	0.0	
3 Standing extraction	Unoiled	0.00	N.D.	0.0	N.D.	0.0	

to pass a 4 mm sieve prior to the addition of waste lubricating oil at concentrations 1:10, 1:40, 1:50 and 1:80 w/w (oil:soil). A spade was used to randomly sample approximately 5 kg of Templeton silt loam top soil (top 12 cm) from a site south of Templeton (grid reference, New Zealand Lands and Survey Department, N.Z.M.S.1 S83/872507). The top soil comprises a very dark grey, very friable A12 horizon (7.5 - 20.0 cm) of medium-fine nutty and cast granule structures and abundant roots overlain by 7 cm of a very dark brown friable silt loam tightly bound by roots and having a strongly developed nutty and cast granular structure and organic matter content of approximately 4.6 percent (New Zealand D.S.I.R. Soil Bureau -Bulletin 14). The Templeton soil was similarly prepared and mixed with waste lubricating oil to provide the same range of oil: soil concentrations. Air-dried Timaru and Templeton soils to which no oil had been added served as controls. Soil moisture contents were 6 and 7 percent respectively. To obtain a quantitative relationship between oil concentration and recovery rate additional oil/soil mixtures (1:6, 1:15, 1:25 and 1:62) of Timaru soil were prepared. Other experimental details were the same as for the previous experiment.

Results for the two soils are given in Tables 2 and 3.

The percentage by weight of oil recovered from both soil types decreased with increasing oil concentration. Rate of recovery was higher for Templeton soil for all oil concentrations except 1:80. The lower recovery rate

Table 2: Recovery of waste lubricating oil from Timaru Silt loam by cold extraction in hexane. † Means of 5 replicates.

Ratio oil: soil	Oil applied g/5g soil	Oil yield † g/5g soil	Percent † oil recovered	C.V.
Unoiled control	0.00	N.D.	00.0	0.0
1:6	0.71	0.47	66.2	6.9
1:9	0.50	0.33	65.6	6.2
1:10	0.45	0.35	77.8	0.0
1:15	0.31	0.25	80.6	2.5
1:25	0.19	0.16	84.2	6.3
1:40	0.12	0.10	83.3	7.3
1:50	0.10	0.08	80.0	0.0
1:62	0.08	0.07	87.5	0.5
1:80	0.06	0.05	83.3	10.1

Table 3: Recovery of waste lubricating oil from Templeton silt loam by cold extraction in hexane. † Means of 5 replicates.

Ratio oil: soil	Oil applied g/5g soil	Oil yield † g/5g soil	Percent oil recovered	C.V.
Unoiled control	0.00	N.D.	0.00	0.0
1:10	0.45	0.40	88.9	0.0
1:40	0.12	0.11	91.7	0.0
1:50	0.10	0.09	90.0	0.0
1:80	0.06	0.05	83.3	0.0

obtained for Timaru Soil may have reflected the different mechanical composition of the soils. Silt and clay particle size which accounted for a greater proportion of the inorganic fraction of the Timaru Soil as determined visually, slowed filtration and permitted a greater evaporative loss of solvent from the filter. This appeared to cause adsorption of further residual oil to the filter.

Before extraction of oil was used to determine the rate of disappearance of oil from soil it was necessary to determine whether the unrecovered oil had been adsorbed by the soil. Adsorbed oil was considered to be the fraction of oil not able to be removed from soil by the extracting solvent. Loran et al. (1973) mixed oily waste and clay containing oil waste, both of refinery origin, with a "clay loam" for which a description was not given and having immediately Soxhlet extracted (benzene:petroleum ether 1:1) the soils could recover only 56.3 and 20.8 percent respectively of the oil. The composition of the oily waste was not given and should the recovery rate change due to increased adsorption by inorganics with repeated cultivation, interpretation of a time course experiment would become difficult. Observations made during the previous experiment of the present study suggested that most of the oil not recovered by standing extraction was removed from the soil but adsorbed the filters. The amount of oil adsorbed by the soil was determined for selected samples of Timaru soil from the previous experiment.

Cold extracted soil samples were placed in a Soxhlet thimble and extracted with hexane for 4 h. Solvent volume of each pre-weighed extraction flask was 100 ml and the Soxhlets cycled once every 3 min. Solvent was removed as previously described. The flasks were reweighed to 0.01 g $\pm$ 0.005 and the residue weights recorded. Selected samples of the cold extracted Templeton soils were similarly treated and results recorded in Tables 4 and 5.

Minimum total oil recovered after Soxhlet extraction was 99.2 and 97.8 percent for Timaru and Templeton soils respectively, indicating that a negligible amount of oil had been fixed by Timaru and Templeton soils. These results contrast with those of Loran *et al.* (1973). In Loran's experiments the chemical and physical properties of the soil clay fraction with which the waste was mixed may have resulted in fixation of oil by the organic and inorganic components of the soil. It is also possible that a different solvent system would have more effectively extracted the aliphatic and aromatic oil fractions from the soil.

With time, alteration of oil in soil may affect soil structure (increased aggregation) which may affect the filtration rate of soil extracts. The ratio of standing: Soxhleted oil fractions was used for a field experiment to detect any changes in the recovery rate of oil removed by cold extraction. Any such changes would be obscured only where a change in recovery rate affects the two fractions similarly.

Table 4 : Recovery of waste lubricating oil from Timaru silt loam by Soxhlet extraction with hexane. † Means of 3 replicates.

Ratio oil: soil	Oil applied g/5g soil	Oil yield† g/5g soil	Percent oil recovered; cold extracted + Soxhlet extracted
Unoiled control	0.0	N.D.	0.0
1:6	0.71	0.21	95.8
1:15	0.31	0.06	100.0
1:25	0.19	0.03	100.0
1:40	0.12	0.02	100.0
1:62	0.08	0.01	100.0

Table 5 : Recovery of waste lubricating oil from Templeton silt loam by Soxhlet extraction with hexane. † means of 3 replicates.

Ratio oil: soil	Oil applied g/5g soil	Oil yield†	Percent oil recovered; cold extracted + Soxhlet extracted
Unoiled control	0.00	N.D.	0.0
1:10	0.45	0.03	95.5
1:50	0.10	.01	100.0

II. FIELD EXPERIMENT FOR THE DISPOSAL OF WASTE LUBRICATING OIL

(1) Experimental Factors

The object of the field experiment was to determine the maximum rate of disappearance of waste oil hydrocarbons by means of degradation by biological agencies. Davis (1967) considered that the rate of biological degradation is influenced by accessability of the hydrocarbon to micro-organisms, its solubility and dispersion in water, its adsorption by clay or organic matter and the physico-chemical properties and the types and number of adsorptive sites of the soil medium while Van der Linden et al. (1967), Schwendinger (1968) and Ellis et al. (1961) considered the composition and size of the hydrocarbon molecules, constitutive and adaptive enzymes and the physical factors of soil aeration, moisture, temperature and pH to be important. Other evidence suggests that addition of nitrogen and phosphorus to oil contaminated soil boosts the soil microflora population (Johansson 1962). It is conceivable that toxic materials in the waste lubricating oil including solvents, phenolics, detergents and heavy metals and by-products of soil transformation may slow breakdown depending on access to and susceptibility of the microflora.

Waste lubricating oil application rate (0) and tillage duration (T) were chosen as factors for a 5 x 4 factorial field experiment because together they related to all the above factors which were considered important to the biological decomposition of waste oil. The aim of the field

experiment therefore, was to determine the effect of tillage duration on the rate of disappearance of waste lubricating oil added at different rates to Timaru soil. The levels of each factor are given in Table 6 and Figure 1. All treatments were duplicated except O_0T_3, O_1T_3, O_2T_3 and O_4T_3 for which there were four replicates. Treatment plots were arranged randomly within two blocks (25 plots/block).

(2) Description of Field Experimental Area

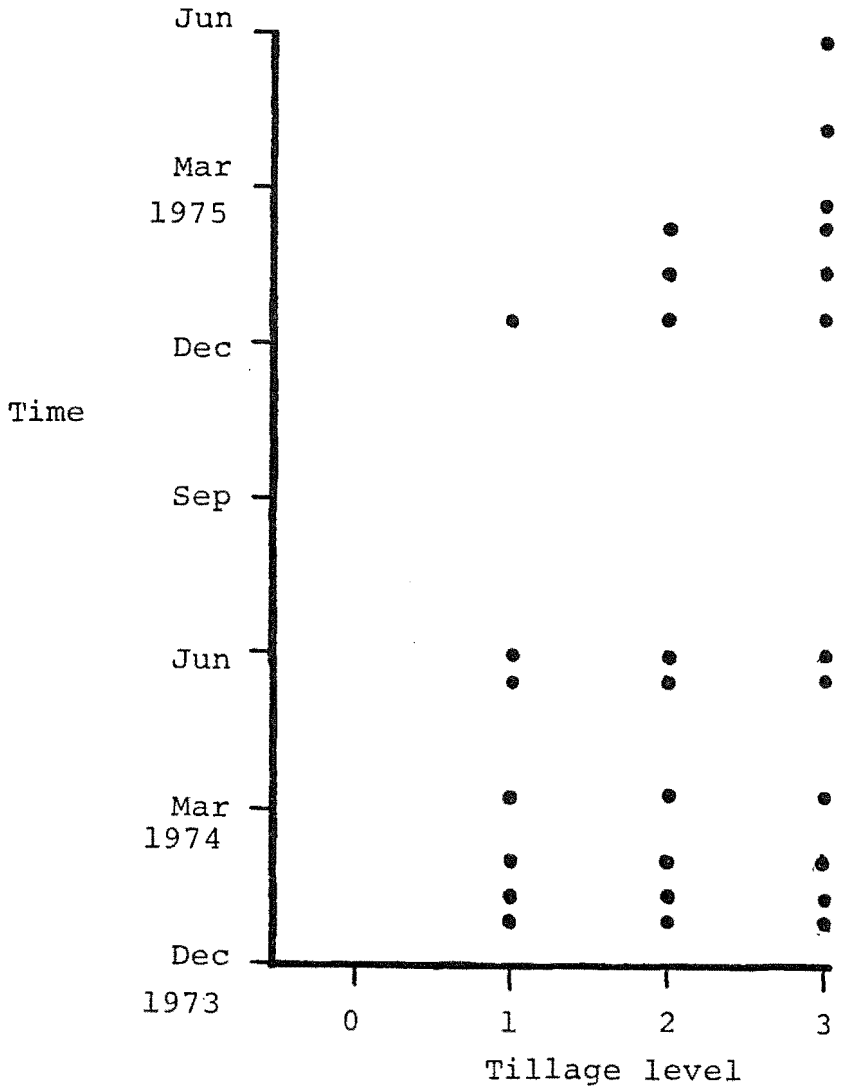
Disposal of city domestic and industrial refuse has traditionally been on suburban land not immediately suited to housing or industrial development. To dispose of a potentially toxic liquid it is desirable that the site have an impermeable sub-soil thereby reducing the risk of leaching and sub-soil contamination. Liquid and by-products may in such cases accumulate immediately above the sub-soil and possibly result in anaerobiosis.

The 0.1 ha area of land chosen at Timaru for the field trial formed part of a low lying (approximately 3m A.S.L.) topographically flat reclaimed estuarine lagoon which lies between parallel shingle field drains. The soil which has already been described, (p.55), supports the growth of predominantly *Agrostis tenuis* and *Poa pratensis* A sp. of *Salicornia* grows in shallow depressions where salts from the wind blown sea spray have accumulated. Annual rainfall is about 50 cm (daily range 0-5.6 cm) and mean annual temperature 11-12^{o}C (annual range 4.8 -

Table 6 : Timaru field trial - waste lubricating oil application rates.

Level	Waste lubricating oil application rate (t/ha)
0	0
1	56
2	112
3	168
4	224

Figure 1 : Timaru field trial-tillage duration.
• Cultivation dates.



37.0°C). Full information concerning rainfall and temperature is given on pp.105 and 106.

(3) Site Preparation

The site was ploughed to a depth of 12 cm early in February 1973 and subsequently rotary cultivated to the same depth using a tractor mounted 'Howard' rotary cultivator of 2.0 m cutting width. Cultivation continued until a maximum clod size of about 2.5 cm was obtained. The pH of the cultivated soil was 5.6 (soil: water 1:2.5). It has been commonly assumed based on plate counts and evidence of hydrocarbon use that bacteria are the most important hydrocarbon decomposers (Dotson et al. (1971) and while the optimum pH for the use of hydrocarbons varies greatly with organisms, a pH near neutral appeared to be most suitable (Ellis et al. 1961). Lime was therefore added (11.5 t/ha) to raise the soil pH to 7. The rate of application was determined using the method of the F.A.O. (Soils Bulletin no. 10). Phosphorus (as superphosphate) was applied at 68 kg/ha to provide a C:P ratio of 10.0:0.1 based on the highest rate of waste oil application and assuming it to have a general formula of C_nH_{2n}. The lime and superphosphate (applied late in July 1973) were mixed and spread with a lime sower of 8.5 m spread. A pH of 7 was obtained for a representative sample of soil (randomly sampled) from the site 16 weeks after lime application. (The method used was that described in the F.A.O. Soils Bulletin no. 10 soil:water 1:2.5 w/v).

(4) Oil Source

Waste lubricating oil from garages serving mainly cars and trucks was collected and stored in a 4.54×10^3 ℓ holding tank near the site of the field trial. An analysis of the oil is given in Appendix 1.

(5) Rate of Oil Application

The application of large amounts of waste lubricating oil to soil in the field may lead to significant losses through surface runoff and leaching. To reduce this risk, the maximum rate of oil application was based on the field capacity for oil of the cultivated soil. Investigations determined the waste lubricating oil holding capacity of soil over a range of soil moisture contents. Approximately 80 g of soil (Timaru Site) which had been raised to water holding capacity was weighed into each of 3 preweighed 250 ml Erhlenmeyer flasks. About 100 g of waste lubricating oil was added to each of the flasks which were then stoppered and shaken on an orbital shaker for 1 h when no further disaggregation of the soil crumb structure appeared likely. Stoppers were removed and the flasks inverted over a muslin strainer. Any free oil drained into pre-weighed beakers. The weight of oil retained /g O.D. soil was computed and the procedure repeated using further soil samples with successively lower moisture contents. Results are given in Table 7.

Table 7: Effect of soil moisture content on weight of oil retained by Timaru silt loam. † Means of 3 replicates.

Percent soil moisture	18.5	12.4	9.6	0.7
g oil retained /g O.D. soil†	0.21	0.22	0.40	0.55

Mokhtar (1973) found that the respiration rate of various soils increased when wetted prior to the application of waste lubricating oil. It was planned to raise the moisture content of the soil to near 10 percent and oil application rates were therefore computed on the basis of the result for 9.6 percent soil moisture content. On the assumption that 1 ha of soil to a depth of 12 cm weighed 1.88×10^6 kg, an oil application rate of 890 t/ha was obtained. To avoid surface runoff of oil during its application and to reduce the risk of leaching, this figure was arbitrarily reduced to one quarter of 890 or 224 t/ha. The other oil application rates - 0, 56, 112 and 168 t/ha were chosen for convenient statistical analysis.

(6) Sub-Soil Permeability to Oil

To determine the permeability to oil of the sub-soil at the Timaru field site, top-soil was removed and 10.1 cm i.d. 12.7 cm long steel cylinders driven into the

sub-soil to a depth of 8 cm. Cylinders and sub-soil were then dug out, placed in plastic bags and returned to the laboratory. Waste lubricating oil was added to the top of the cylinder to provide a 4.7 cm head. After three weeks the oil was poured off and the cores visually inspected. Oil in the cylinders had remained at the same level and did not appear to have penetrated the surface of the clay which was therefore considered impermeable to oil. Top soil was omitted from the experiment to obtain an indication of contamination under extreme conditions.

(7) Method of Oil Application

Rate of oil application was one of the factors under investigation and it was therefore necessary to design equipment by which the correct amount of waste lubricating oil could be applied uniformly over the soil. Plate 2 shows the tractor mounted spray apparatus used for the experiment. Oil from a 200 ℓ drum was pumped through the spray-bar by a 5 cm centrifugal pump powered by the drive from the tractor which was driven at constant rev/min. The amount of oil delivered was checked by reading the oil level on a calibrated clear plastic tube fixed to one end of the drum.

(8) Tillage

Number and dates of cultivation for the various tillage levels are given in Figure 6. On the date of each cultivation

Plate 2: Oil spraying equipment

the plots to be cultivated were grubbed once to a depth of 12 cm using a tractor mounted spring tyne grubber an agricultural implement commonly used to cultivate soil. Forty-five weeks after oil application, a tractor mounted 'Howard' rotary cultivator having a cutting width of approximately 2 m was used instead of grubbing to facilitate better mixing of oil and soil. The cultivation depth was not altered.

(9) Pilot Sampling Experiment

Valid statistical comparison of oil concentration in field plots required a measure of the maximum variability for oil concentration in the soil. A sampling programme could then be established for the main experiment. Three sampling points for a plot area of 1.38 m^2 and five for a plot area of 5.1 m^2 were considered representative by Kincannon et al. (1972) and Raymond et al. (1976) respectively. Points for the former study were selected systematically but sampling details were not given by Raymond.

A pilot experiment was marked out on the cultivated site at Timaru, tilled once with a spring-tyne grubber to a depth of 12 cm and oil applied at a rate of 40t/ha. The soil was then grubbed a second time to mix in the oil. A low waste lubricating oil application rate of 40 t/ha was chosen so that an upper limit to oil concentration variability over a plot could be determined. The plot was divided into three equal strata and two 12 cm cores taken with a plastic corer at each of 9 sampling

points selected randomly from each stratum. The first set of cores from each stratum were combined to give three samples (a plot total of 9) while those of the second set were stored separately. All samples were placed in labelled plastic bags and prepared and extracted in hexane as previously described. The statistical F values obtained for between strata/within strata were 0.42 and 0.30 for individual and combination methods respectively. Both were non significant and the coefficient of variation was therefore substantially reduced by combining samples. Using the following formula (after Sokal and Rohlf 1969), the number of cores required for each plot to be 80 percent certain of detecting a 75 percent difference between any two treatment means at the 5 percent level of significance was computed as shown below

$$\text{Using } n \geqslant 2\left(\frac{\sigma}{\delta}\right)^2 \{t_1\ \alpha[\nu] + t_2(1-p)[\nu]\}^2$$

where C. of V. = 104 percent

$\alpha = 0.05 \qquad n = 60$

$\nu = 25(60-1) = 1475$

$\sigma = \frac{104\ \bar{Y}}{100}$

δ to be 60 percent of the mean.

$$\text{i.e. } \delta = \frac{60\ \bar{Y}}{100}$$

Using δ as an estimate of σ

$$\frac{\sigma}{\delta} = \frac{\left(\frac{104\ \bar{Y}}{100}\right)}{\left(\frac{60\ \bar{Y}}{100}\right)} = 1.73$$

$$\{t_1\ \alpha[\nu] + t_2(1-p)[\nu]\}^2$$

$$= 9.73$$

$$\text{Then } n \geqslant 2[(1.73)^2(9.73)]$$

$$\geqslant 2(29.09)$$

$$= 58.18$$

(10) Data Presentation

Several methods may be used to prepare samples for oil extraction. Kincannon et al. (1972) heated samples of oily soil at 105°C for 1 h prior to extraction and expressed results in terms of oven-dried soil. Soil samples of different oil concentration may dry off at different rates to give non uniform recovery rates. Sampling of the present field experiment within 7 d of a fall of rain was avoided because of difficulties in

mixing sub-samples from each field plot. Samples were air-dried prior to cold extraction and results expressed as g oil /5g of air-dried soil. The method was acceptable because there were no large differences in the water content of soils from different oil treatments and soils from different sampling dates.

(11) Sensitivity of Method

Assuming that 1 ha of soil to a depth of 12 cm weighs 1.88×10^6 kg and an oil detection limit of 0.01 g/5g soil, undetected oil in the field would be 4 t/ha or 7.1, 3.6, 2.4 and 1.8 percent for 56, 112, 168 and 224 t/ha oil application rates respectively. Lesser amounts of oil could be detected by increasing the weight of the soil sample in which case a further oil recovery experiment would be necessary.

(12) Effect of Tillage Depth on Oil Concentration

Repeated cultivation of field plots of oiled soil may result in some variation in tillage depth. A high soil moisture content may permit the cultivator to sink further into the ground thereby increasing the depth of cultivated soil. The oil concentration of the soil would be diluted by the extra soil resulting in an artificially high rate of oil loss. The composition of an oiled soil can be expressed as follows:

$$S = O + NOM + P + M + B$$

S = oiled soil

O = extractable oil

NOM = native organic matter

P = products of microbial alteration

M = moisture

B = ignited extracted soil residue†

The weights of residue obtained after extraction and ignition of oiled soil samples of the same weight should be approximately equal where additions of waste lubricating oil were small, successive cultivations made to the same depth and other things being equal. A waste oil application rate of 224 t/ha would increase the weight of a soil volume (1 ha to a depth of 12 cm*) by 10 percent, the maximum percentage increase expected for B assuming the oil alteration products are lost from the soil by leaching or evaporation. A greater increase would indicate significant increase in tillage depth. Mean weight of ignited extracted soil residue was therefore measured for each soil sampling during the field trial in order that corrections be made to the oil concentration of the soil where necessary.

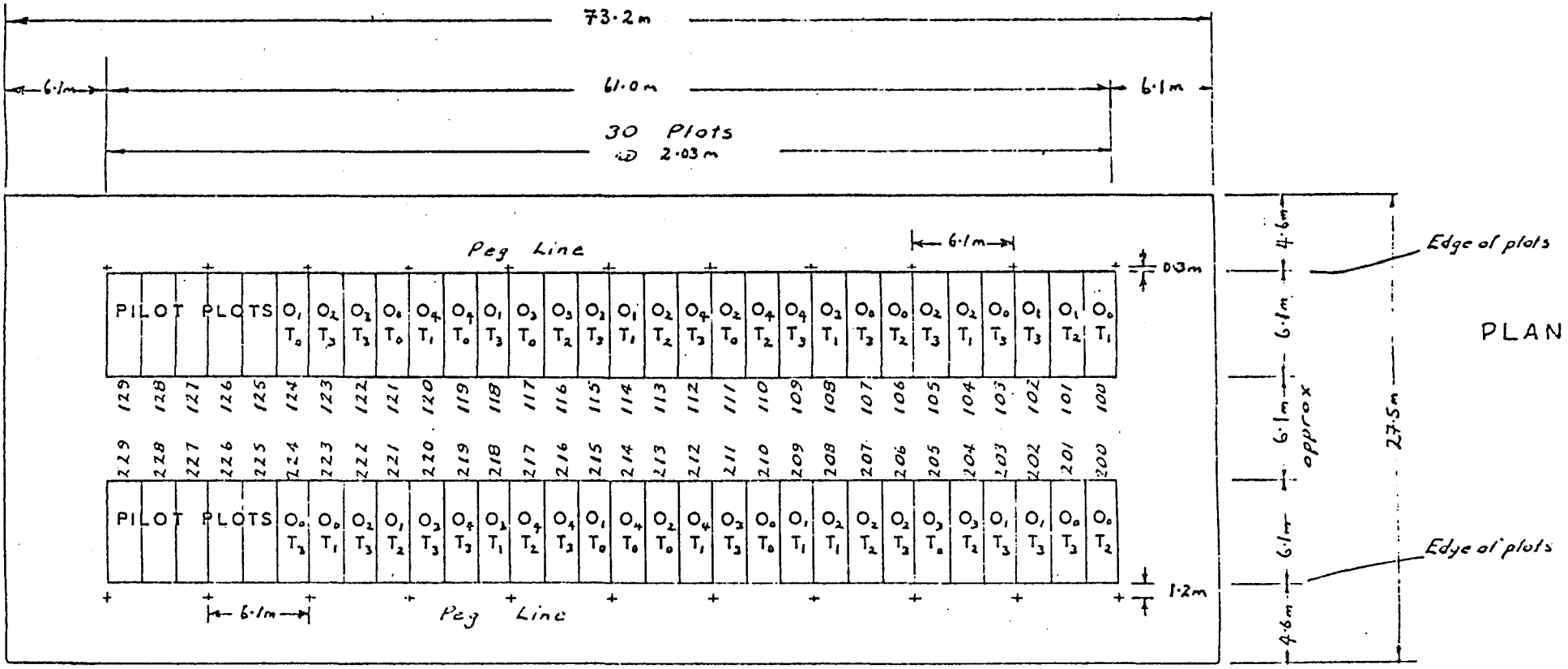
(13) Main Field Plots

Layout of the field experiment is shown in Fig. 2. Plots were separated by a 0.55 m border to prevent oil contamination. Waste lubricating oil was applied on

† Oiled soil sample extracted with hexane and ignited in a muffle furnace at 380°C for 16 h to remove organic matter.- A waste lubricating oil control was not included because only relative yields were of interest.

* A soil volume assumed to weigh 1.88×10^6 kg

Figure 2: Plan of Timaru field experimental plots, Saltwater Creek. Grid ref. New Zealand Topographical Map N.Z.M.S.1 S111 Timaru 784488. Scale, 1 cm equals approximately 1.92 m.



December 7-8, 1973. A tanker fitted with a spray bar was used to spread water over both blocks at a rate of 44 t/ha. The rate was based on that required to raise the soil moisture content to approximately 10 percent. Oil was then applied by tractor as earlier described and mixed with the soil to a depth of 12 cm using a spring-tyne grubber. Plates 3 and 4 show the soil surface before and after oil cultivation.

Four weeks after oil application, 60 core samples were taken from each plot using randomly selected pairs of co-ordinates. Alternate cores were placed in two labelled 550 ml, 9.0 cm i.d. 'Agee' jars (Crown Crystal Glass, subsidiary of Alex Harvey Industries Ltd., Shands Road Hornby, Christchurch 4, New Zealand) to reduce the number of analyses, returned to the laboratory, air-dried and ground in a mortar with a pestle to pass a 4 mm sieve. The soil consistency was such that prior spreading was unnecessary. Three 5 g sub-sub-samples were removed from each jar (sub-sample) and cold extracted as described elsewhere. Each soil extract weight for this and subsequent samplings was converted to a value corresponding to 100 percent recovery. The conversions were made directly from a linear regression line (Fig. 3) obtained for an earlier soil recovery experiment (p.60).

A nested analysis of variance was performed to test sampling efficiency. Results are given in Table 8.

The highly significant F values and percentage variance obtained for the first three samplings suggested that more representative sampling would improve sampling efficiency.

Plate 3: Field plot 219 after oil application 224 t/ha and before cultivation

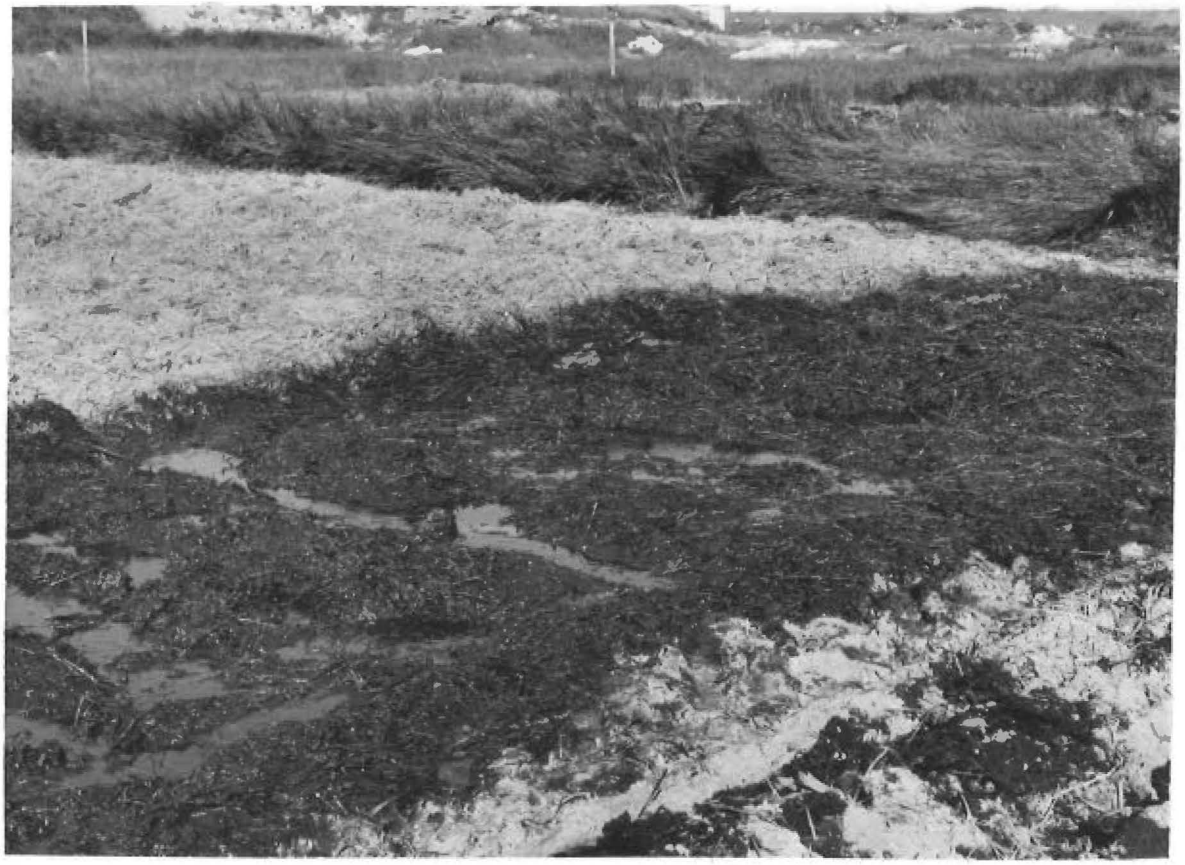
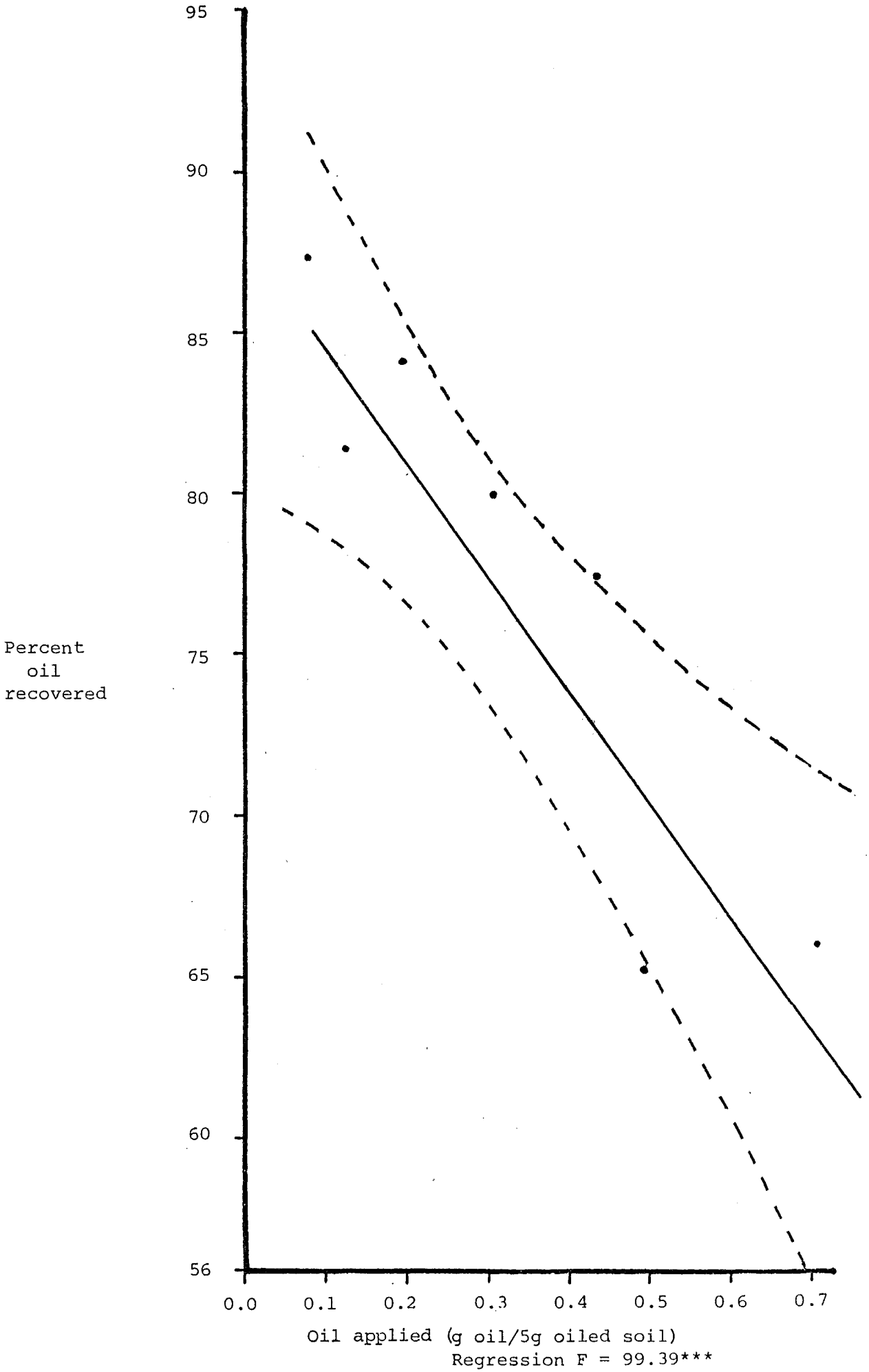


Plate 4: Field plot 219 cultivated subsequent to oil application at 224 t/ha

Figure 3: Percentage oil recovered from Timaru silt loam containing oil at a range of concentrations.



Equation for line is Y = 88.25 - 35.11X. Bands represent 95 percent confidence limits to the regression line.

Table 8: Oil content of Timaru field plots 4 weeks after oil application (sampling 1). Nested analysis of variance.

Source	d.f.	F	Percentage variance
Between oil treatments	3	30.5***	70.5
Between blocks within oil treatments	36	9.0***	21.5
Between sub-samples within oil treatments	40	4.1***	4.1
Between sub-sub-samples within sub-samples	159	-	3.9

Table 8a : Three way analysis of variance.

Source		d.f.	F
Blocks	B	1	1.9
Oil	O	3	122.8***
Tillage	T	3	10.2***
	BO	3	3.5*
	BT	3	4.7**
	OT	9	4.4***
	BOT	9	3.2**

The results of a second (three way analysis of variance) analysis for the first sampling are shown in Table 8a.

The highly significant F values obtained for the factor tillage and first and second order interactions indicatedthat the sampling method was inadequate. Data for all plots of each application rate and block were expressed as histograms to obtain information on possible trends in oil concentration (Figs. 4 & 5). Numerical order of plots on each histogram is the same as that in the field (see p.77). Oil concentration for soils of most oil application rates of both experimental blocks increased from south (low plot number) to north suggesting that the results may have been affected by some soil gradient. Cores removed during the first sampling were all taken to the same depth but pieces of undecomposed plant matter in the soil made cores of uniform volume difficult to obtain. The plots had been cultivated only once after oil application and perfect mixing of soil throughout the soil volume could not therefore be expected. Any gradient of oil concentration down the soil profile would therefore lead to anomalous soil extract weights unless cores of consistent volume were sampled.

To investigate the possibility that variations in the soil organic matter content of different plots was responsible for the observed pattern of oil concentration in the experimental blocks, the organic matter content of the unoiled control plots of both blocks was determined

Figure 4: Oil content of east block Timaru field plots 4 weeks after oil application (sampling 1)

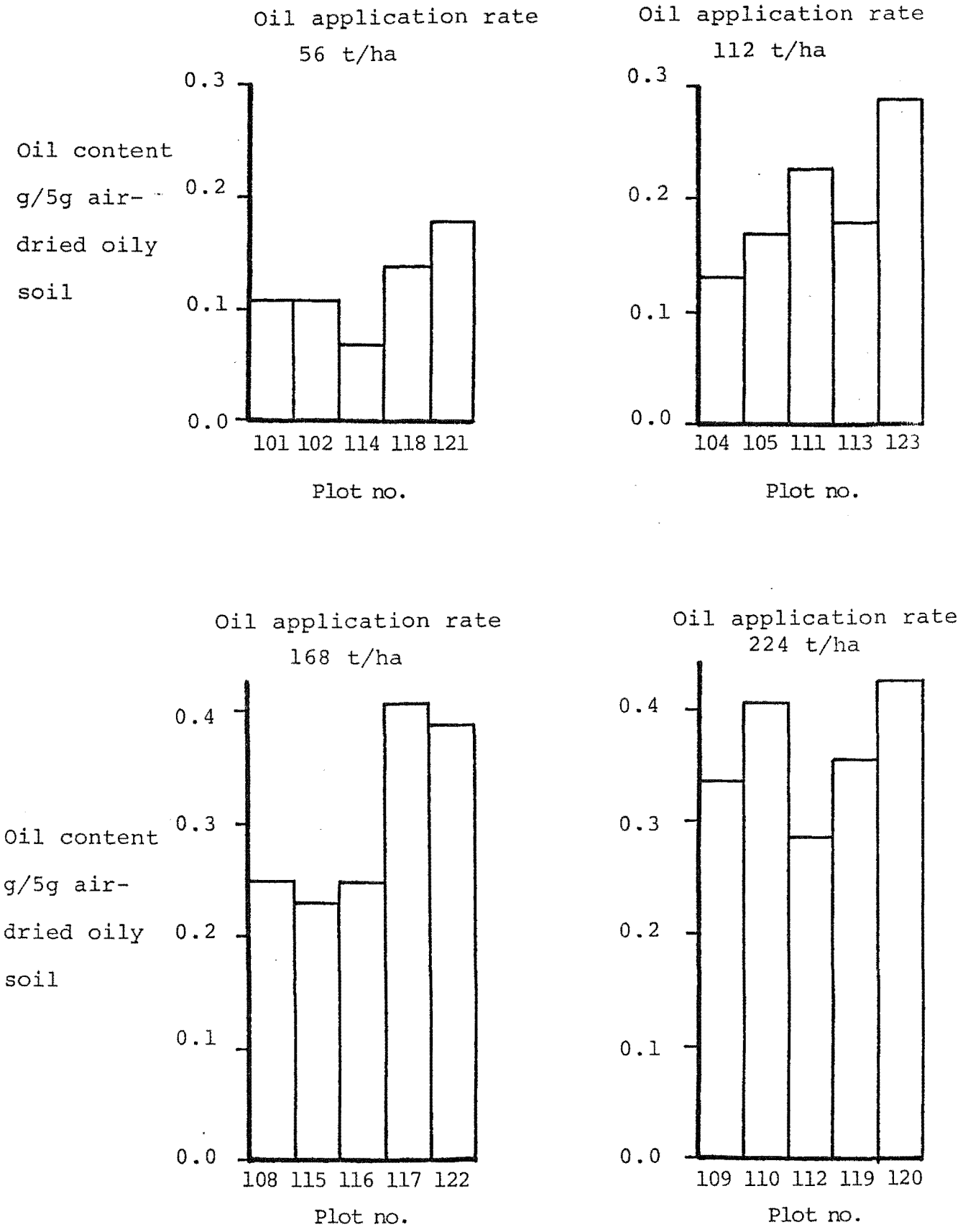
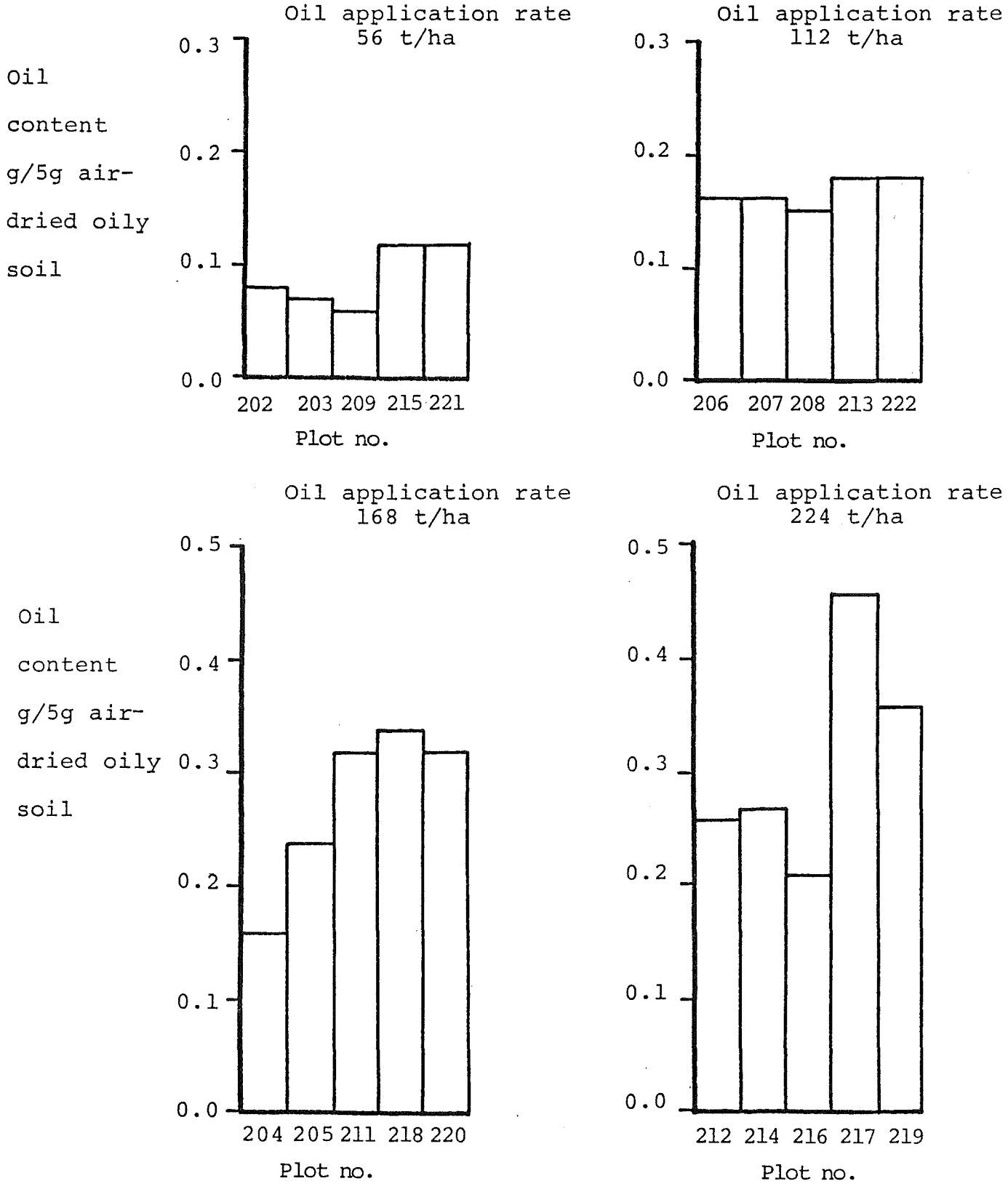


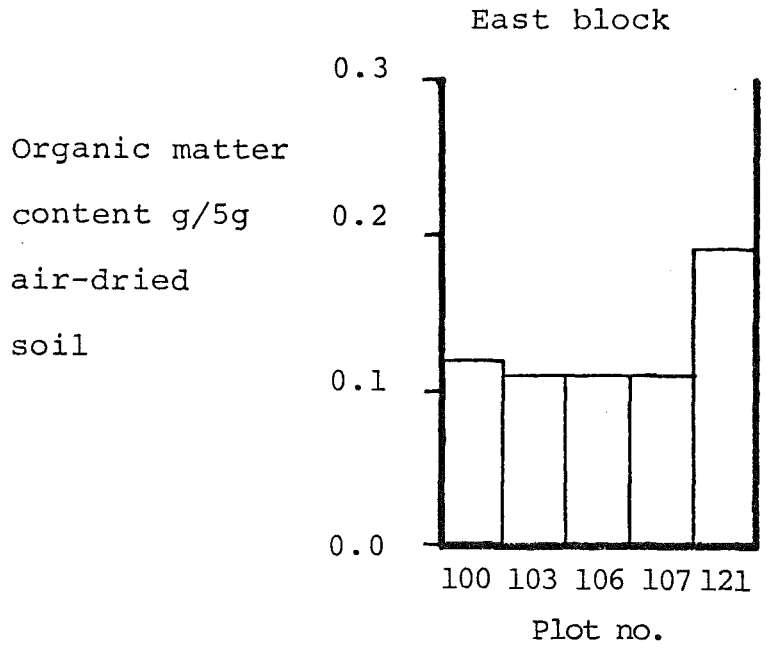
Figure 5: Oil content of west block Timaru field plots 4 weeks after oil application. (sampling 1).

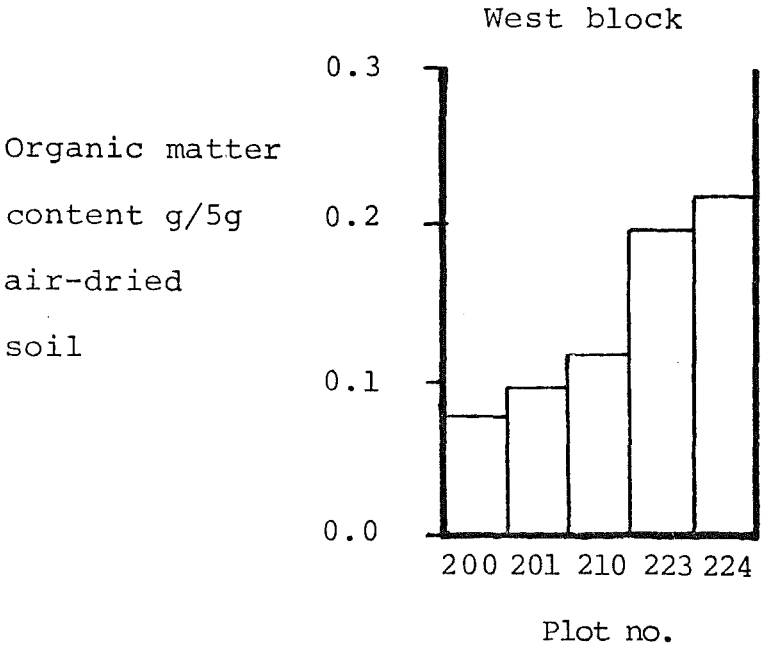


and plotted as histograms (Figure 6). Soil organic matter was taken to be the weight loss upon ignition at 380°C for 16 h (Ball 1964) of oven-dried hexane extracted soil samples. The soil organic matter content of both experimental blocks increased from south to north and was most marked in the West Block where the unoiled control plots were more evenly distributed along the block. A similar observation was made concerning oil concentration. Ignited extracted residue weight was approximately the same for all soils. In addition to its effect on core consistency, the variation in soil organic matter content may have affected the oil recovery rate for soil of the experimental blocks. The results of an earlier experiment indicated a different oil recovery rate for Timaru and Templeton soils and this was presumed due to differences in particle size distribution. Conceivably, differences in organic matter content had a similar effect. An experiment was therefore carried out to investigate the possibility that the recovery rate of waste lubricating oil mixed with Timaru soil was affected by soil organic matter content.

One kg of soil (randomly sampled) was removed from each of the 200 and 224 plots, air-dried and ground in a mortar with a pestle to pass a 4 mm sieve. Waste lubricating oil was mixed with 30 g samples of each soil to provide soil:oil concentrations of 6:1, 15:1, 25:1, 40:1 and 62:1. Samples weighing 5 g $\pm$ 0.05 were cold extracted in hexane as previously described. Results presented in Table 9 show similar percentage recovery of added oil for both

Figure 6: Organic matter content of Timaru unoiled field plots 4 weeks after oil application (sampling 1).





soils at all oil concentrations. Thus, extremes of organic matter content found in Timaru plots did not appear

Table 9: Effect of soil organic matter content on percentage oil recovered from Timaru silt loam, 4 weeks after oil application (sampling 1).
† Means of 5 replicates.

Field Plot	Percentage oil recovered †				
	Ratio soil:oil				
	6:1	15:1	25:1	40:1	62:1
200	71.1	80.9	79.6	86.8	82.9
224	72.3	81.9	79.6	86.8	85.4

to have affected the recovery rate of waste lubricating oil.

A different sampling method was used to make a second sampling of the field area on March 8, 1974, 12 weeks after oil application. A trowel was used to take 60 random samples from each of the field plots. The samples were taken to a depth of 12 cm and placed in a 48 ℓ bucket which was shaken vigorously to mix the soil. Two 550 ml 'Agee' jars were used to remove two samples from the bucket and the remainder returned to the plot. Samples were prepared and extracted as for sampling 1. Nested and 3-way analyses of variance of the data are given in Tables 10, 10a, 10b and 10c.

Table 10 : Oil content of Timaru field plots 12 weeks after oil application (sampling 2). Nested analysis of variance.

Source	d.f.	Fs	Percentage variance
Between oil treatments	11	31.7	90.1
Between blocks within oil treatments	20	6.6	6.6
Between sub-samples within treatments	32	5.2	1.9
Between sub-sub-samples	128		1.4

Table 10a: Three way analysis of variance.

Source		D.F.	F.
Blocks	B	1	0.0
Oil	O	3	529.5***
Tillage	T	3	0.3
	BO	3	1.7
	BT	3	0.1
	OT	9	5.4*
	BOT	9	2.1

Table 10b: Main effect - oil

Oil yield g/5g air dried soil.

Oil application rate (t/ha)				
0	56	112	168	224
N.D.	.15	.37	.42	.52

S.E. = ± 0.010488 L.S.D. (5%) = 0.03

Table 10c: Interaction O x T.

Oil yield - g/5g air dried soil.

Tillage level	Oil application rate (t/ha)				
	0	56	112	168	224
0	N.D.	0.15	0.28	0.43	0.51
1	N.D.	0.12	0.28	0.43	0.55
2	N.D.	0.17	0.24	0.43	0.51
3	N.D.	0.17	0.29	0.40	0.50

S.E. = ± 0.020926 L.S.D. (5%) = 0.06

Inspection of the percentage variance for each level of the nested analysis of variance shows an increase of 20 percent attributable to between treatment differences compared to that obtained for the first sampling. The significant interaction between oil application rate and tillage level is difficult to explain because with the exception of the untilled plots all had received the same number of cultivations. That 90 percent of the variance of the nested analysis was attributable to between treatment differences indicated more representative sampling and the significant oil x tillage interaction obtained was therefore presumably due to variations in the rate of oil applied to the field plots.

Inadequate sampling technique made it difficult to determine accurately the oil content of the field plots 4 weeks after oil application. Oil losses occurring during the 12 weeks elapsed since the time of oil application were therefore estimated from the mean weight of oil obtained for each of the five oil application rates 12 weeks after oil application. Oil weights were converted to t oil/ha based on the assumption that 1 ha of soil to a depth of 12 cm weighed 1.88×10^6 kg (Table 11). The data indicated an increased percentage loss with increasing oil application rate. Variation obtained for oil concentration of the 224 t/ha plot suggested that inaccurate application rates may have been partly responsible for the lower values obtained for plots of this application rate. Such variation may have arisen because of tractor slip during later passes over an already oil saturated soil.

Table 11: Estimated oil losses from Timaru field plots 12 weeks after oil application. Means of 60 samples

	Oil application rate (t/ha)				
	0	56	112	168	224
Oil remaining 12 weeks after sampling (t/ha)	N.D.	58	101	158	188
Percentage oil remaining 12 weeks after oil application	0	104	90	94	84

Nitrogen as calcium ammonium nitrate was applied to all plots at a rate of 225 kg/ha on November 13, 1974, 45 weeks after oil application. It was hoped that by delaying application until the summer season, leaching losses would be reduced. The single application was made as evenly as possible to each plot by hand spreading during north-south and east-west traverses. To obtain better mixing of the oil and soil it was decided to use a rotary cultivator instead of a grubber to cultivate the soil plots (see p. 72).

Four further samplings were carried out over the next 28 weeks using the same procedure as described for sampling 2. Analysis of these results are recorded in Tables 12, 13, 14

and 15. Loss of oil from the soil through time is shown in Figures 7, 8 and 9. Data for plot oil concentrations prior to 12 weeks after oil application, the time of the second sampling,were not available because the first sampling method was found to be inadequate. To avoid soil disturbance, the zero tillage plots were excluded from samplings 3, 4 and 5. Average rates of oil loss as t/ha/month were computed from treatment mean oil concentrations (g/5g air dried soil) which had been corrected for 100 percent recovery (p. 56) and converted to t/ha on the assumption the 1 ha of soil to a depth of 12 cm weighed 1.88×10^6 kg.

From the evidence given in Figures 7,8,9,10, and Tables 16 and 17, oil was lost from soil plots of all treatments throughout the field trial and in the absence of cultivation the mean rate of disappearance increased with oil application rate up to 168 t/ha. Short term cultivation of the oiled soil (tillage level 1), increased the average rate of oil loss for all application rates except 56 t/ha and was most effective for applications of 168 and 224 t/ha. Further tillage (level 2) increased the rate of diaappearance from both 56 and 224 t/ha plots but was ineffective at intermediate rates. An increased rate of oil loss with additional cultivation (tillage level 3) was observed only for 224 t/ha plots. The maximum rate of oil disappearance for tillage levels 1, 2, and 3 occurred between samplings 3 and 5 (November 1974 - February 1975). Sampling 3 coincided with the

Table 12 : Oil content of Timaru field plots 45 weeks after oil application. (Sampling 3)

Three way analysis of variance.

Source		d.f.	F
Blocks	B	1	2.0 n.s.
Oil	O	3	297.3***
Tillage	T	2	2.2 n.s.
	BO	3	1.7 n.s.
	BT	2	1.4 n.s.
	OT	6	4.9***
	BOT	6	1.2 n.s.

Table 12a : Main effect - oil.

Oil yield g/5g air-dried soil.

Oil application rate (t/ha)				
0	56	112	168	224
N.D.	0.07	0.17	0.24	0.35

S.E. = ± 0.008778 L.S.D.(5%) = 0.03

Table 12b : Interaction O x T

Oil yield g/5g air-dried soil.

Tillage level	Oil application rate t/ha				
	0	56	112	168	224
1	N.D.	0.59	0.17	0.24	0.36
2	N.D.	0.76	0.16	0.21	0.37
3	N.D.	0.74	0.16	0.26	0.31

S.E. = ± 0.014876 L.S.D. (5%) = 0.04

Table 13 : Oil content of Timaru field plots 52 weeks after oil application, (sampling 4). Three-way analysis of variance.

Source		d.f.	F
Blocks	B	1	0.4
Oil	O	3	309.0***
Tillage	T	2	13.7***
	BO	3	2.2
	BT	2	0.1
	OT	6	3.7***
	BOT	6	1.7

Table 13a : Main effect - oil

Oil yield g/5g air-dried soil.

Oil application rate t/ha				
0	56	112	168	224
N.D.	0.06	0.14	0.19	0.26

S.E. = ± 0.006249 L.S.D. (5%) = 0.02

Table 13b : Main effect - tillage.

Oil yield g/5g air-dried soil.

Tillage level		
1	2	3
0.21	0.20	0.21

S.E. = ± 0.005391 L.S.D. (5%) = 0.01

Table 13c : Interaction O x T.

Oil yield g/5g air-dried soil.

Tillage level	Oil application rate t/ha				
	0	56	112	168	224
1	N.D.	0.05	0.16	0.21	0.29
2	N.D.	0.06	0.12	0.17	0.24
3	N.D.	0.07	0.13	0.19	0.24

S.E. = ± 0.010283 L.S.D. (5%) = 0.03

Table 14: Oil content of Timaru field plots 58 weeks after oil application (sampling 5). Three-way analysis of variance.

Source		d.f.	F.
Blocks	B	1	0.9
Oil	O	3	255.0***
Tillage	T	2	22.3***
	BO	3	1.5
	BT	2	0.2
	OT	6	15.4***
	BOT	6	0.4

Table 14a : Main effect - oil.

Oil yield g/5g air-dried soil.

Oil application rate t/ha				
0	56	112	168	224
	0.04	0.11	0.15	0.21

S.E. = ± 0.005916 L.S.D. (5%) = 0.02

Table 14b : Main effect - tillage.

Oil yield g/5g air-dried Soil.

Tillage level		
1	2	3
0.19	0.17	0.15

S.E. = ± 0.005235 L.S.D. (5%) = 0.01

Table 14c : Interaction O x T.

Oil Yield g/5g air-dried soil.

Tillage level	Oil application rate t/ha				
	0	56	112	168	224
1	N.D.	0.04	0.12	0.15	0.26
2	N.D.	0.05	0.12	0.15	0.21
3	N.D.	0.05	0.09	0.14	0.15

S.E. = ± 0.010248 L.S.D. (5%) = 0.03

Table 15 : Oil content of Timaru field plots 71 weeks after oil application (sampling 6). Three-way analysis of variance.

Source		d.f.	F
Blocks	B	1	2.7
Oil	O	3	639.8***
Tillage	T	3	209.8***
	BO	3	2.6
	BT	3	4.8**
	OT	9	52.1***
	BOT	9	4.3***

Table 15a: Main effect - oil.

Oil yield g/5g air-dried soil.

Oil application rate t/ha				
0	56	112	168	224
N.D.	0.04	0.08	0.13	0.20

S.E. = ± 0.003464 L.S.D. (5%) = 0.01

Table 15b: Main effect- tillage.

Oil yield g/5g air-dried soil.

Tillage level			
1	2	3	4
0.16	0.12	0.09	0.08

S.E. = ± 0.003464 L.S.D. (5%) = 0.01

Table 15c : Interaction O x T

Oil yield g/5g air-dried soil

Tillage level	Oil application rate t/ha				
	0	56	112	168	224
0	N.D.	0.05	0.11	0.19	0.31
1	N.D.	0.03	0.08	0.12	0.23
2	N.D.	0.03	0.07	0.11	0.16
3	N.D.	0.03	0.07	0.10	0.10

S.E. = ± 0.006928 L.S.D. (5%) = 0.02

Figure 7: Oil loss from Timaru field plots, tillage level. Oil concentration for the unoiled controls was less than the detection limit. Unlinked points, uncultivated controls • ± S.E. of the mean.

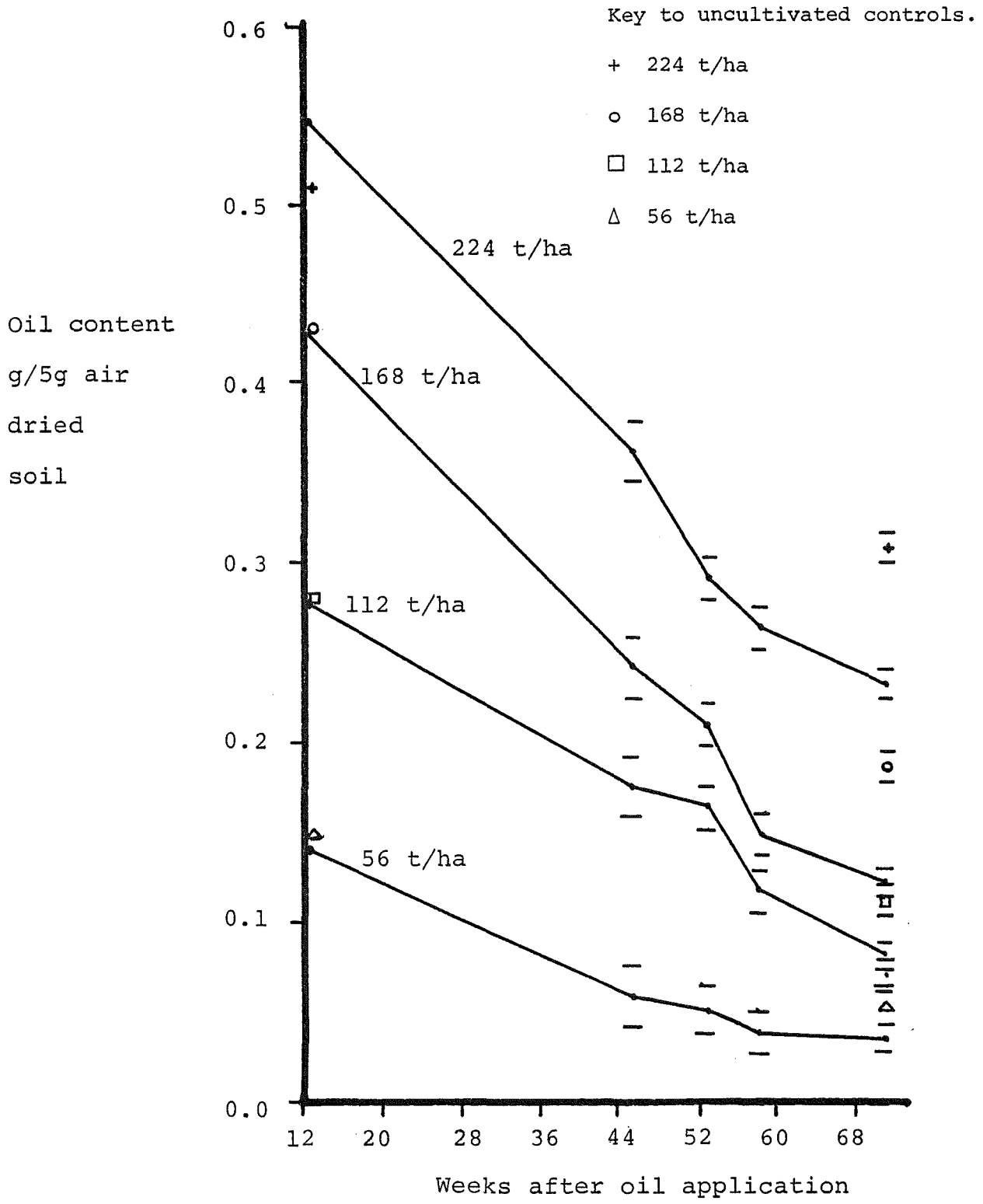


Figure 8: Oil loss from Timaru field plots, tillage level 2. Oil concentration for the unoiled controls was less than the detection limit. Unlinked points, uncultivated controls.
— ± S.E. of the mean.

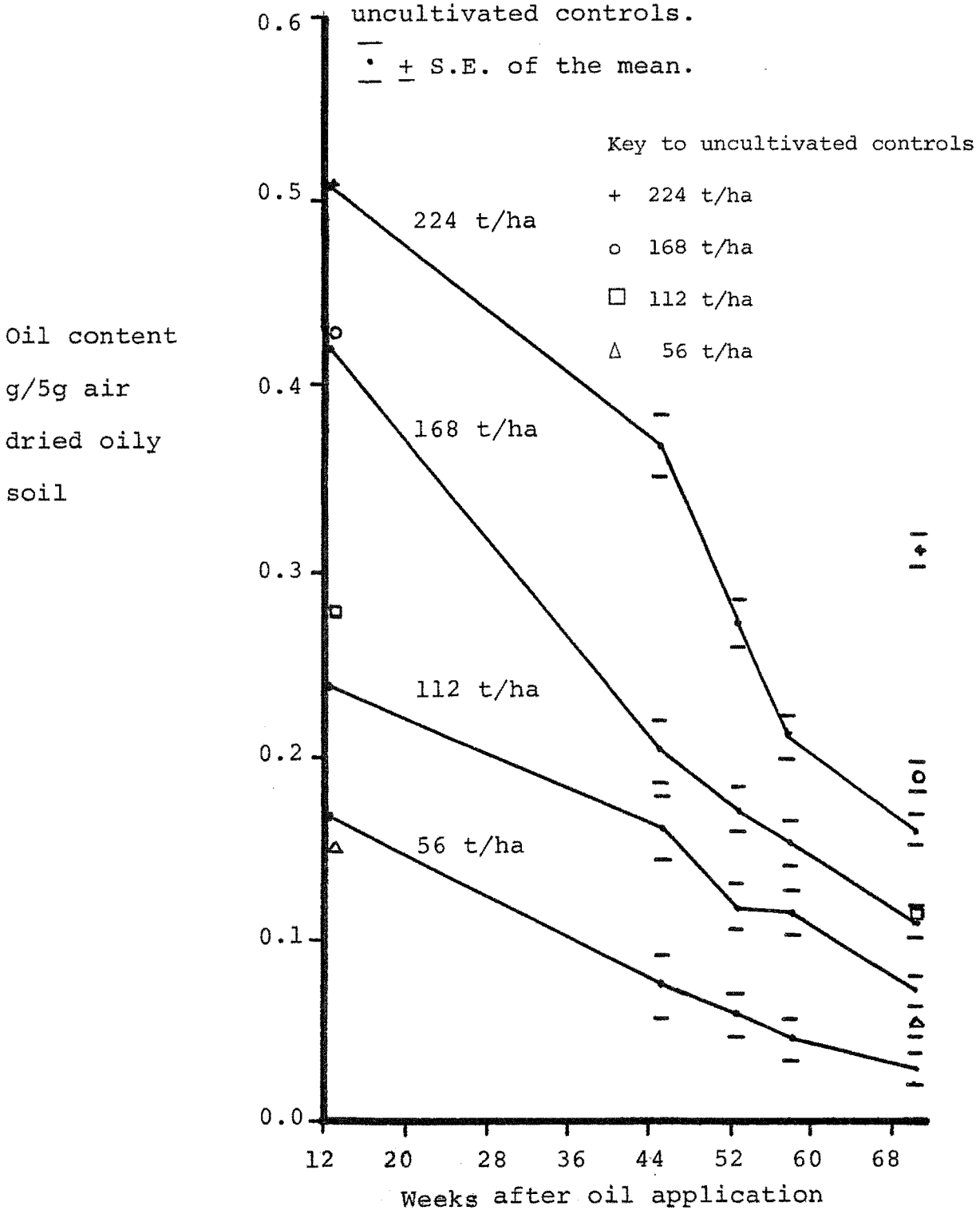


Figure 9: Oil loss from Timaru field plots, Tillage level 3. Oil concentration for the unoiled controls was less than the detection limit. Unlinked points, uncultivated controls.
$\bar{\cdot}$ ± S.E. of the mean.

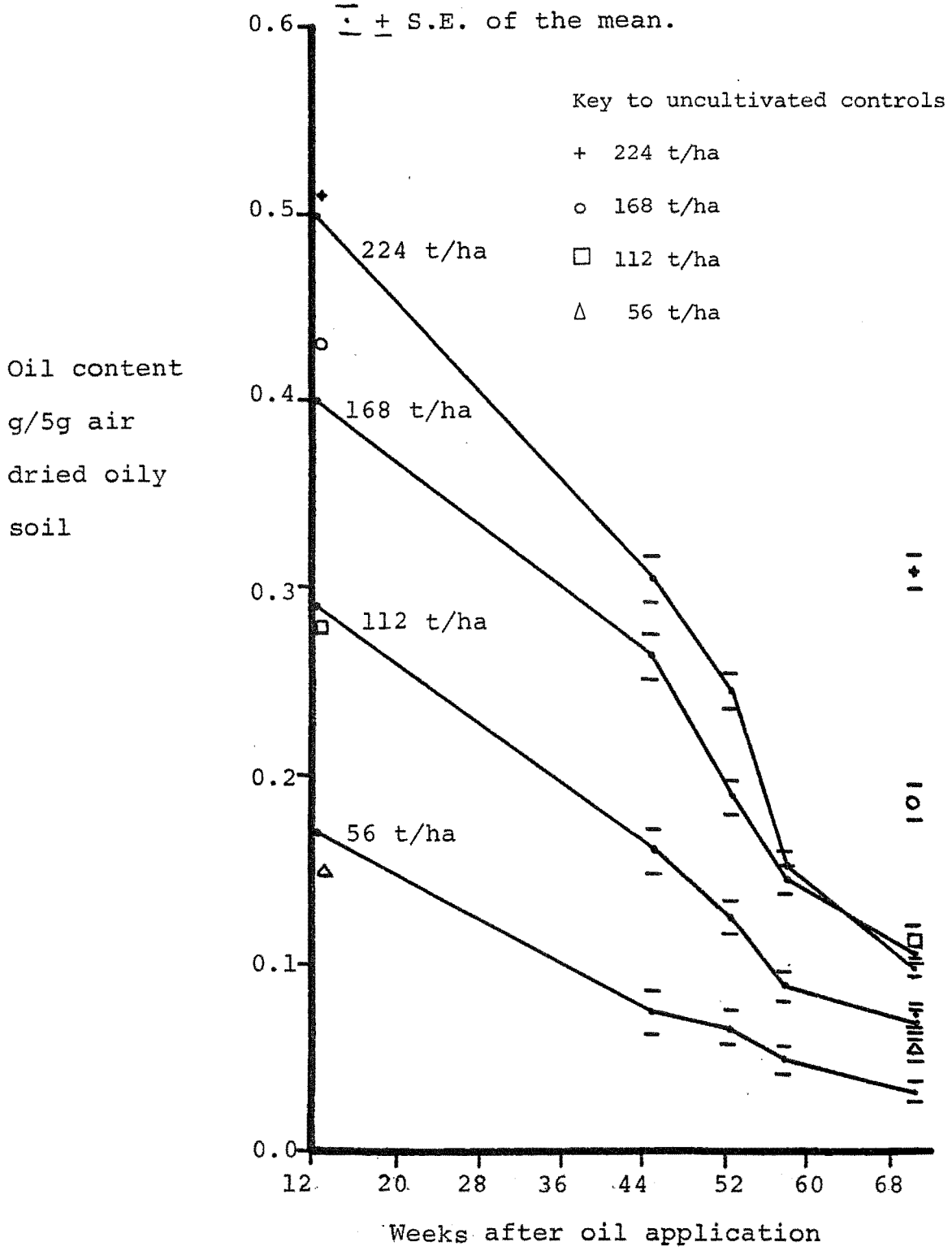


Figure 10: Effect of oil application rate and tillage duration on the rate of loss of waste lubricating oil from Timaru silt loam.

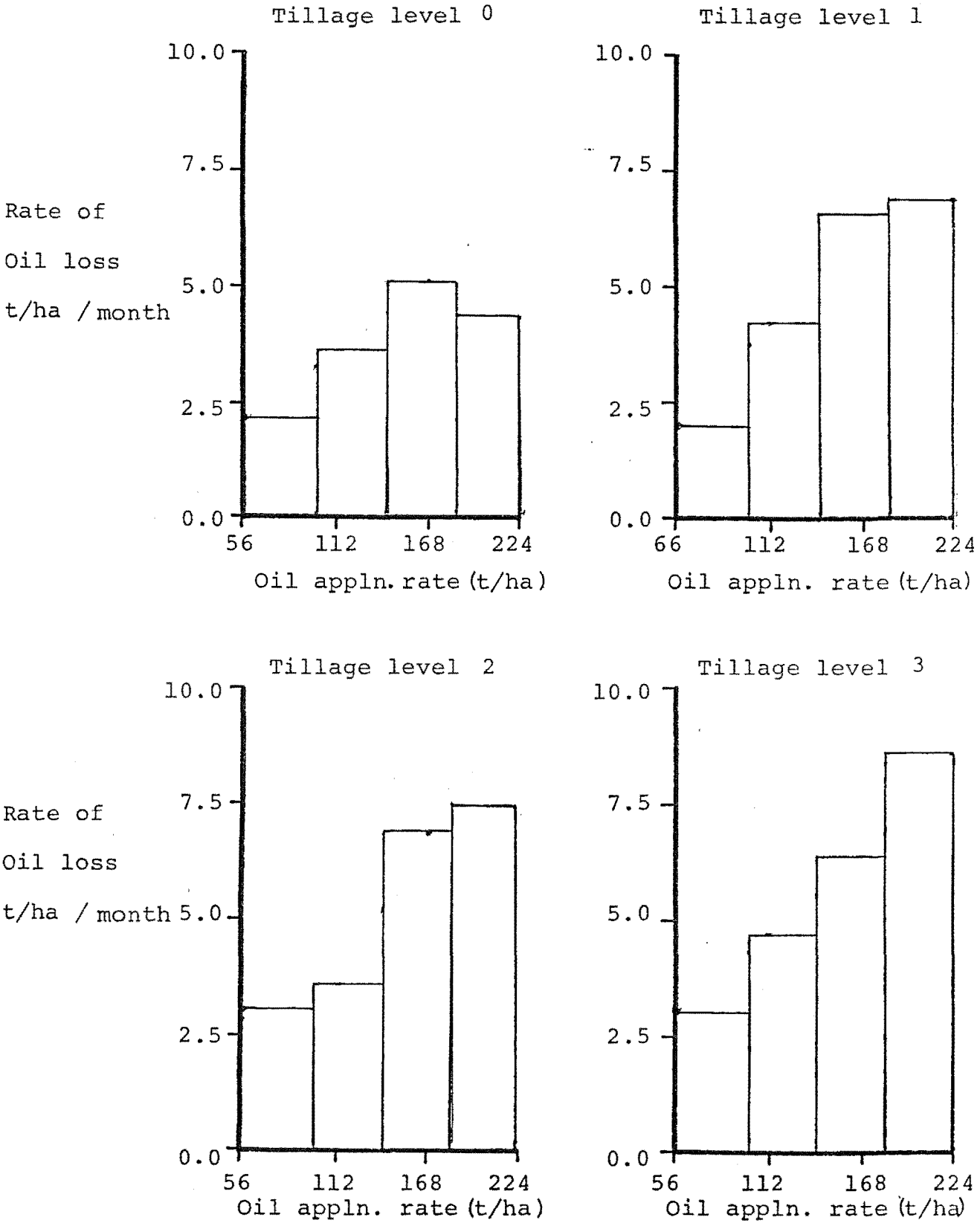


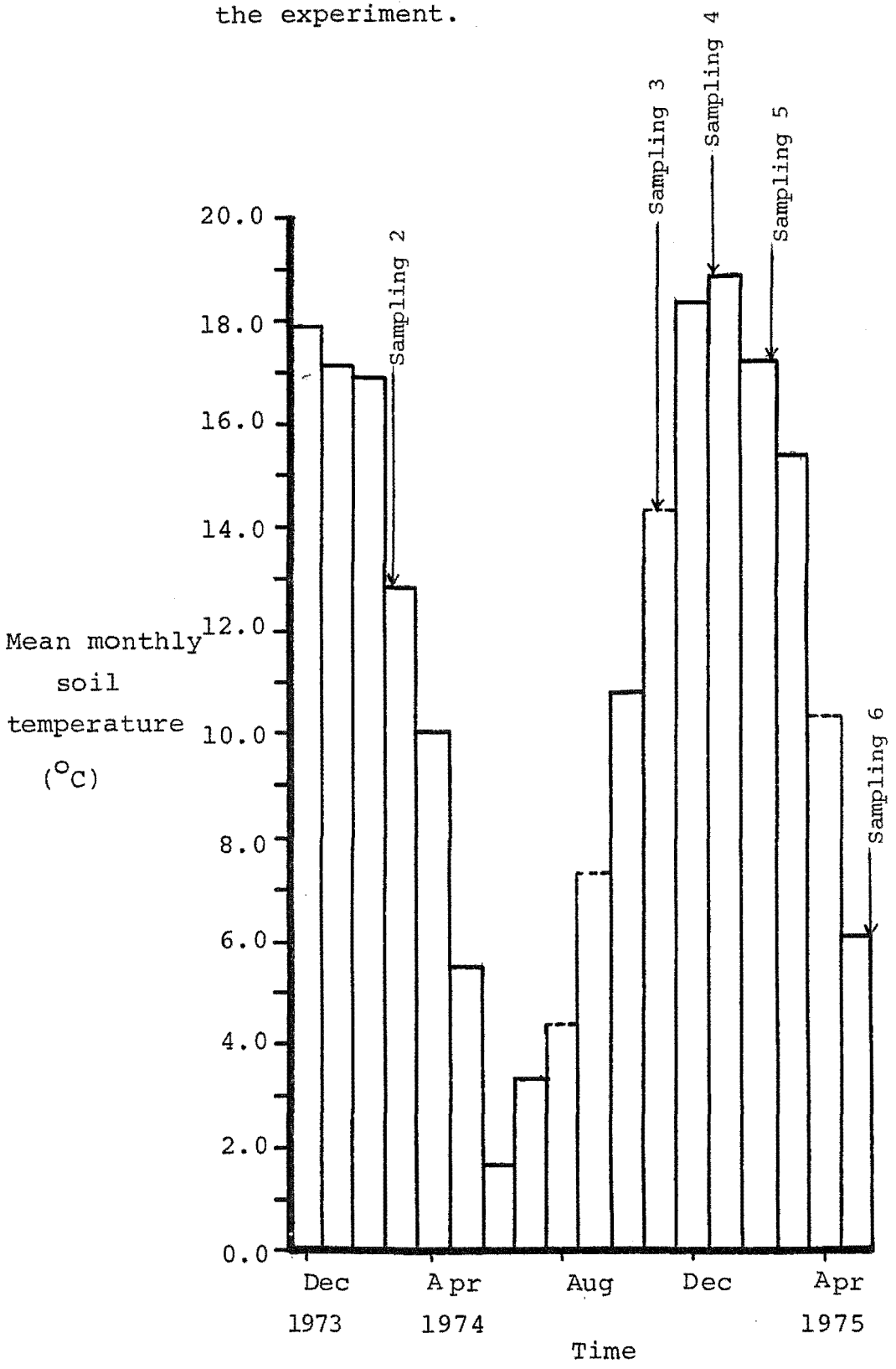
Table 16: Rate of oil loss from Timaru silt loam t/ha/month

Tillage level	Oil application rate t/ha			
	56	112	168	224
0	2.1	3.6	5.1	4.3
1	1.9	4.3	6.6	6.9
2	3.0	3.6	6.9	7.5
3	3.0	4.7	6.4	8.6

Table 17: Residual oil in Timaru silt loam 71 weeks after oil application (t/ha)

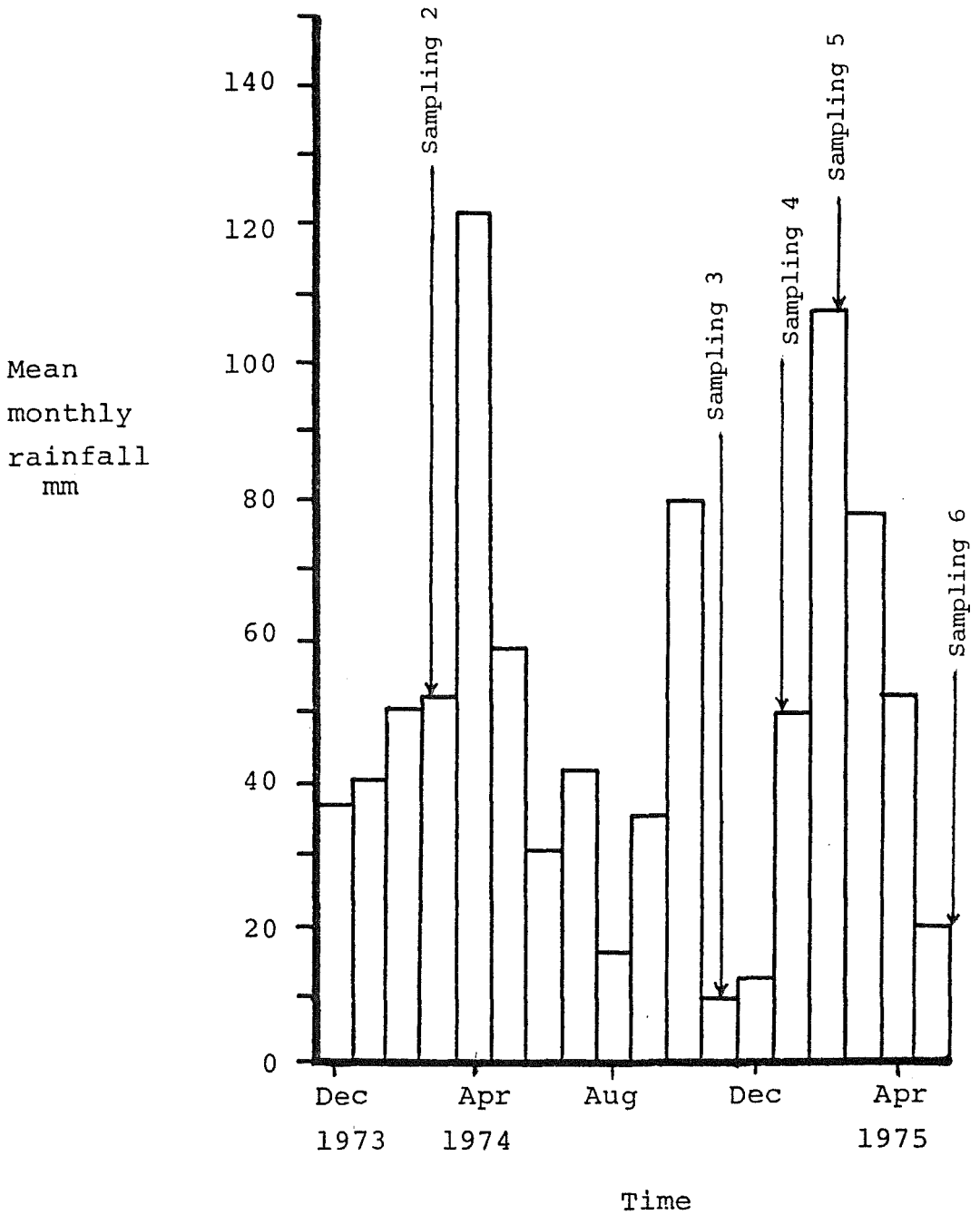
Tillage level	Oil application rate t/ha			
	56	112	168	224
0	19.0	41.8	72.2	117.8
1	11.4	30.4	45.6	87.4
2	11.4	26.6	41.8	60.4
3	11.4	26.7	38.0	37.6

Figure 11: Soil temperature - Timaru (depth 0.1 m)
Based on data from Ministry of Transport New Zealand Meteorological Service for Timaru botanic gardens 17 m A.S.L. and approximately one mile from the site of the experiment.



Broken line denotes months for which data was not available.
Means represent averages of appropriate monthly means from the preceeding and the following year.

Figure 12: Rainfall - Timaru. Based on data from Ministry of Transport New Zealand Meteorological Service for Timaru botanic gardens 17 m A.S.L. and approximately one mile from the site of the experiment.



application of nitrogen and a change from grubbing to rotary cultivation. There were relatively high soil temperatures, (17.3°C-19.0°C Figure 11) during these four summer months and monthly rainfall (Figure 12) increased from 10 mm (October) to 50 and 108 mm for the months of January and February respectively. Reduced average rates of waste oil disappearance from all plots followed between samplings 5 and 6, months for which both rainfall and soil temperature were lower.

(14) Ratio Standing: Soxhleted Extract Fractions - Main Field Plots.

As can be seen from Table 18, results for field plots selected to include high and low oil application rates indicated no significant departures from 12 to 71 weeks (samplings 2-6) after oil application.

Table 18: Ratio percentage oil cold extracted: percentage oil Soxhleted extracted

Weeks after oil application	Plot no.			
	212	120	209	102
12	85.7:14.3	85.3:14.7	85.7:14.3	85.7:14.3
71	87.5:12.5	83.3:16.7	83.3:16.7	82.3:17.7

(15) Ignited Extracted Soil Residue - Main Field Plots

Results for weight of ignited extracted soil (g/5g air dried soil) from samplings 2 to 6 (Table 19) indicated that residue weight did not change greater than approximately 5 percent which was within experimental error. On the basis of this evidence tillage depth did not significantly increase between samplings 2 and 6.

Table 19: Ignited extracted residue from Timaru field plots, g/5g air dired oil. † Means of all treatments and replicates

	Sampling				
	2	3	4	5	6
g ignited extracted soil residue/5g air-dried soil†	4.58	6.61	4.72	4.72	4.70

(16) Oil Losses in Drainage Water

Total monthly rainfall during the first three months following application at Timaru did not exceed 50 mm (Figure 12). This period corresponded to relatively high monthly mean soil temperatures (12°C-18°C) (Figure 11) and ponding of water did not occur on the soil plots. The highest monthly rainfall (122 mm) was recorded for the next month of the field trial (April 1974) and coincided

with lower soil temperatures (10.1°C).

The experimental area was almost level and water therefore remained on the surface of the near saturated soils of the field plots for the following two months. Although appearing drier than the unoiled controls, the soil of all plots was very sticky and made cultivation impossible. On May 19, 1974, 22 weeks after oil application, a bull-dozer was used to excavate drainage channels at the border of each experimental block. The channels (approximately 10 cm deep) connected with a 0.6 m deep ditch dug to meet a west-east draining stream at the northern end of the experimental area. Runoff water from the plots was negligible until October 1974 when a monthly rainfall maximum of 81 mm and daily maximum of 29 mm were recorded. No oil was visible on the surface of the runoff water suggesting such losses were small. To determine the extent of these losses, three 1 ℓ water samples were taken from each drainage channel on October 10. All samples were collected within 1 h and taken by intercepting the water flow with 9 cm i.d. 1 ℓ 'Agee' jars.

Oil extraction followed the procedure given by the Environmental Protection Agency 1971:- 0.01 g $\pm$ 0.005 of waste lubricating oil was added to a 1 ℓ jar filled with distilled water. The sample was then acidified to a pH below 3. A filter, prepared from a muslin cloth disc overlain with filter paper (Whatman's no. 1) was placed in a Buchner funnel and the filter paper wetted. One

hundred ml of filter aid suspension (diatomaceous earth) was added to the filter and washed three times with 100 ml of distilled water under vacuum. After vacuum filtration of the acidified sample, the filter paper was removed from the funnel with a pair of forceps and placed in a Soxhlet thimble. Sides and bottom of the jar, stirring rod and Buchner flask were wiped with pieces of filter paper and soaked in hexane. The extraction thimble containing the filter paper and pieces of filter used for cleaning was oven-dried at 105°C for 30 min and extracted with hexane into a preweighed flask at a rate of 20 c/h for 4 h. Solvent was removed from the extract with a vacuum rotary evaporator and dried to constant weight using a silica gel filtered air stream. Extract weights were computed from the flasks reweighed to $\pm$ 0.005 g. Two further samples were extracted on a vacuum pump and the procedure repeated for additions of 0.03 g waste lubricating oil and no oil. Results for the recovery experiment and field samples are given in Tables 20 and 21.

The area of land occupied by the field plots was about 723 m^2. Total daily rainfall on the day of sampling was about 0.03 m. Assuming that the soil immediately prior to the rainfall had been saturated with water, a runoff volume on the day of sampling of about 21.6 m^3 would be obtained. Mean weight of extract from the runoff samples was 14.3 mg/ℓ which, assuming constant oil content for the runoff water corresponded to a total loss for the day of 309 g which represented less than 3.2×10^{-5} percent of the oil originally added.

Table 20: Percentage waste lubricating oil recovered from water by Soxhlet extraction with hexane. † Means of 3 replicates. C.V. = 6.2 percent.

	Weight oil added		
	0.00	0.01	0.03
Mean percent oil recovered †	N.D.	97	96

Table 21: Hexane extractable material from runoff water of the Timaru field area. Extract weight mg/ℓ.

Drainage channel sampled	Sample				
	1	2	3	mean	C.V.
East block	11	11	21	14.3	6.5
West block	22	10	11	14.3	6.7

(17) Oil Contamination of the Sub-Soil

Sub-soil permeability experiments (p. 69) had shown that the sub-soil at the Timaru field site was impermeable to waste lubricating oil suggesting that under field conditions such losses would be small.

Eighty weeks after oil application (July 1975) the cultivated 12 cm of top soil was removed from each of the oiled plots and samples of the sub-soil taken for oil content determination. Three sites were randomly selected from each plot and using a spade, 0.02 m^2 of top-soil was removed from each site. The top 1 cm of sub-soil was scraped off and placed in separate labelled bags. All samples were returned to the laboratory, air-dried, ground in a mortar with a pestle to pass a 4 mm sieve and extracted using a Soxhlet as previously described. A separate oil recovery experiment was performed using sub-soil from one of the unoiled control plots. Waste lubricating oil was added to sub-soil to provide concentrations of 0.01 g oil/5g air-dried sub-soil. One hundred percent recovery was obtained from the three Soxhlet extracted sub-samples. The extract weight obtained from field scrapings and cores was less than the detection limit suggesting that sub-soil losses due to leaching during the field trial had been negligible.

(18) Evaporative Losses of Waste Lubricating Oil from Main Field Plots

Measurement of evaporative losses of waste lubricating oil from soil under field conditions was made difficult by

the problems of obtaining and maintaining in the field, sterile samples of oily soil. An experiment was therefore carried out in vitro under extreme conditions to determine loss of waste lubricating oil by evaporation.

Five kg of soil was randomly sampled from an unoiled plot (103) of the Timaru field experiment, returned to the laboratory and air-dried. One hundred g $\pm$ 0.1 of the air-dried soil and 11 g $\pm$ 0.1 of waste lubricating oil from the tank used to supply the Timaru field experiment were added to each of three preweighed 250 ml beakers and mixed thoroughly with a glass rod. Three other preweighed 250 ml beakers containing oiled 100 g $\pm$ 0.1 of soil served as a control. The six beakers were placed in an oven at 105°C and reweighed 5 d later. Weight loss recorded for the oil treated soil (means of 3 replicates) was only 4.5 percent greater than that obtained for the control suggesting that evaporative losses of waste lubricating oil under less extreme conditions would have been small.

(19) Vegetative Cover on Main Field Plots

The dominant plants at the Timaru field trial site before oil application were *Agrostis tenuis* and *Poa pratensis* which, during site preparation were mixed with the soil by ploughing and rotary cultivation. A few weeks after oil application, regrowth of the two species was observed on both unoiled and oiled plots.

An arbitrary scale of 1-3 was used to record vegetative cover on the field plots 16 weeks after oil application

(29/11/74) when cultivated plots had received an equal number of cultivations. As the field trial progressed, fewer plots were cultivated and for the purpose of comparison when a second recording was made 50 weeks after oil application, only uncultivated plots were included. Results (Table 22) show that for both blocks the percentage cover increased from south (low plot number) to north. No obvious relationship between plant cover and oil application rate was observed. Excepting plot 111 (56 t/ha), near complete cover was established on the uncultivated east block plots. Establishment of plant cover on uncultivated west block plots proceeded more slowly. Photographs were taken of the uncultivated field plots 26 and 53 weeks after oil application to show plant cover on soils to which different amounts of oil had been applied. Percentage cover and sward height as determined visually 26 weeks after oil application on the oiled plots of the east block was similar to that of the unoiled control except for plot 111 (112 t/ha) which had less growth (Plates 5, 7, 9, 11 and 13). Percentage cover recorded on the east block plots for 168 and 224 t/ha oil application rates 16 weeks after oil application (Table 22) suggested that oil residues were not responsible for the poorer growth recorded on plot 111. Taller plant cover at the eastern end of plot 119 (224 t/ha) may have reflected variation in oil concentration over the plot. The pattern of growth for these plots was similar 53 weeks after oil application (Plates 6, 8, 10, 12 and 14). Plates 15 to 23 show growth on the west block uncultivated plots. Percentage

Table 22: Vegetative Cover on Timaru Field Plots.

Plot no.	Oil application rate (t/ha)	Cover east block* arbitrary scale		Plot no.	Oil application rate (t/ha)	Cover west block* arbitrary scale	
Cultivated plots		16 weeks after oil application	50 weeks after oil application	Cultivated plots		16 weeks after oil application	50 weeks after oil application
100	0	0	-	200	0	0	-
101	56	0	-	201	0	1	-
102	56	0	-	202	56	1	-
103	0	0	-	203	56	0	-
104	112	0	-	204	168	0	-
105	112	0	-	206	112	0	-
106	0	0	-	207	112	0	-
107	0	0	-	208	112	0	-
108	168	0	-	209	56	0	-
109	224	0	-	211	168	0	-
110	224	0	-	212	224	0	-
112	224	0	-	216	224	2	-
113	112	0	-	217	224	2	-
114	56	1	-	218	168	2	-
115	168	2	-	219	224	2	-
116	168	1	-	220	168	2	-
118	56	2	-	221	56	2	-
120	224	1	-	222	112	2	-
122	168	1	-	223	0	2	-
123	112	1	-	224	0	2	-
Uncultivated plots							
111	112	1	2	205	168	0	2
117	168	3	3	210	0	2	3
119	224	2	3	213	112	1	2
121	0	3	3	214	224	2	2
124	56	3	3	215	56	3	3

* 1 < percent cover
2 10-70 percent cover
3 70-100 percent cover

Plate 5: Plant cover on plot 121, 26 weeks after oil application at 0 t/ha.

Plate 6: Plant cover on plot 121, 53 weeks after oil application at 0 t/ha.

Plate 7: Plant cover on plot 124, 26 weeks after oil application at 56 t/ha.

Plate 8: Plant cover on plot 124, 53 weeks after oil application at 56 t/ha.

Plate 9: Plant cover on plot 111, 26 weeks after oil application at 112 t/ha.

Plate 10: Plant cover on plot 111, 53 weeks after oil application at 112 t/ha.

Plate 11: Plant cover on plot 117, 26 weeks after oil application at 168 t/ha.

Plate 12: Plant cover on plot 117, 53 weeks after oil application at 168 t/ha.

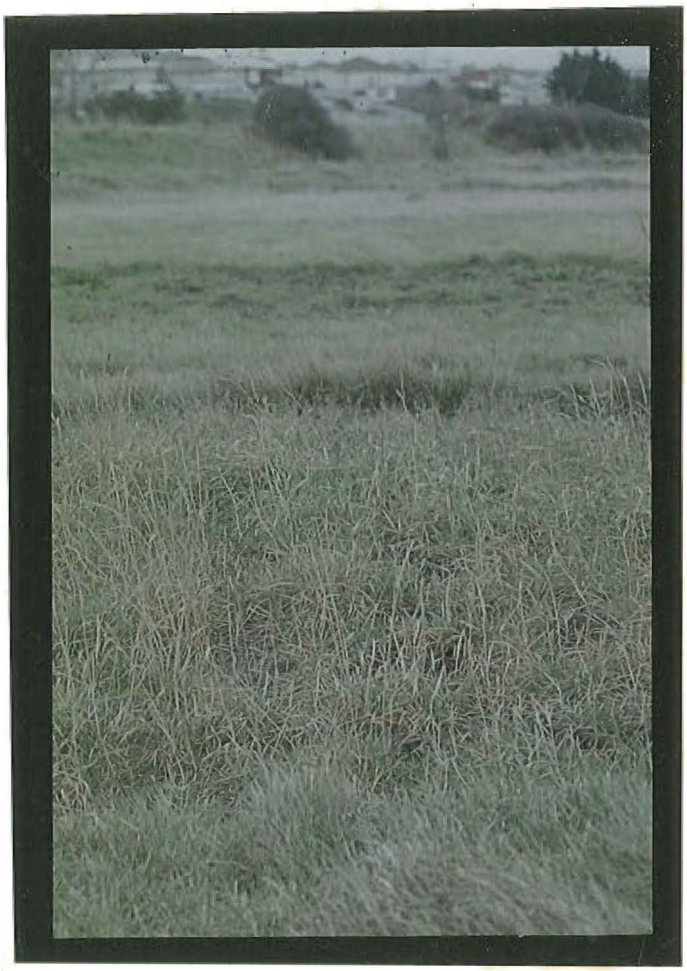


Plate 13: Plant cover on plot 119, 26 weeks after oil application at 224 t/ha.

Plate 14: Plant cover on plot 119, 53 weeks after oil application at 224 t/ha.

Plate 15: Plant cover on plot 210, 26 weeks after oil application at 0 t/ha.

Plate 16: Plant cover on plot 210, 53 weeks after oil application at 0 t/ha.

Plate 17: Plant cover on plot 215, 26 weeks after oil application at 56 t/ha.

Plate 18: Plant cover on plot 215, 53 weeks after oil application at 56 t/ha.

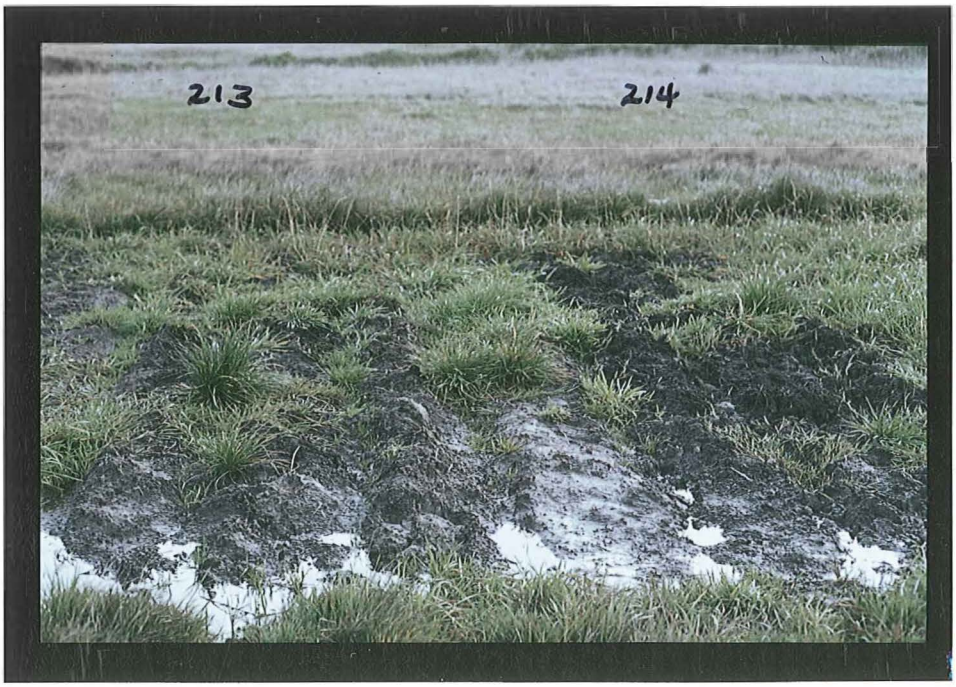


Plate 19: Plant cover on plots 213-214, 26 weeks after oil application at 112 t/ha and 224 t/ha respectively.

Plate 20: Plant cover on plot 213, 53 weeks after oil application at 112 t/ha.

Plate 21: Plant cover on plot 214, 53 weeks after oil application at 224 t/ha.

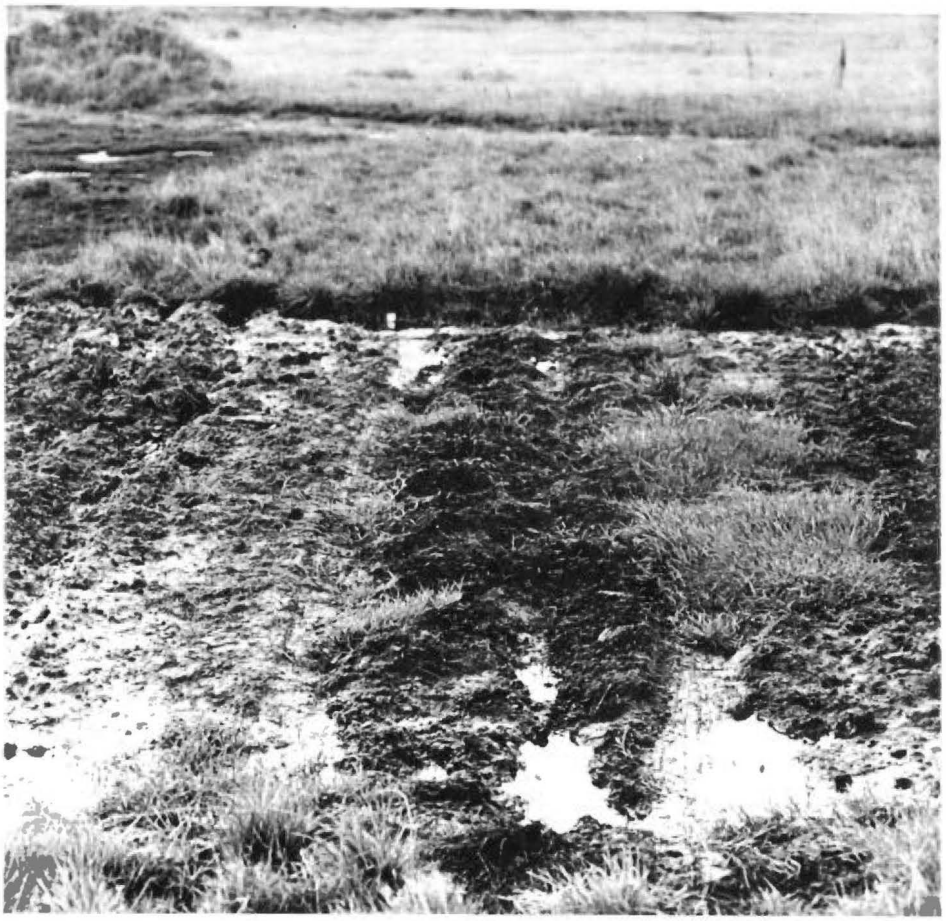


Plate 22: Plant cover on plot 205,
26 weeks after oil application,
at 168 t/ha.

Plate 23: Plant cover on plot 205, 53 weeks after oil application at 168 t/ha.

cover 26 weeks after oil application was generally less than that on the east block equivalents and sward height not as great as 53 weeks after oil application.

(20) Appearance of Soil after Oil Application

Immediately following oil application, ponding of oil was observed on plots receiving 112, 168 and 224 t/ha (Plate 3) but disappeared after the oil and soil had been mixed once with a spring-tyne cultivator (Plate 4). All the oiled plots were noticeably darker than the unoiled controls. Soil was darkest for oil application rates of 224 t/ha. Plate 19 shows the colour difference between plots of 112 t/ha and 224 t/ha oil application rates. As the field trial progressed, the colour of the cultivated oiled plots became lighter but at its conclusion, visual differences were still present. Although some fading of the darker soil of the uncultivated oiled plots did occur, colour differences at the conclusion of the field trial were greater than those observed for the cultivated plots. Darker soils of the oiled plots were considerably warmer than those of the unoiled controls on the hot sunny days indicating that waste lubricating oil may have had a black-body radiator effect on the soil.

A preliminary experiment was carried out to determine the effect of waste lubricating oil addition on soil temperature. Ninety eight weeks after oil application, two kg of soil was randomly sampled from an unoiled plot (103) at

the Timaru experimental site, returned to the laboratory and air-dried. Eleven g ± 0.1 of waste lubricating oil from the tank used to supply the field experiment was mixed with 100 g ± 0.1 of soil in a 250 ml glass beaker to provide an oil concentration of approximately 10 percent w/w which was approximately the same as that of the 224 t/ha field oil application rate. A second beaker containing only 100 g ± 0.1 of soil served as a control. Treatment and control were replicated twice and a thermometer was placed in the centre of each pot such that the base was 1 cm from the soil surface. The pots were placed in a glasshouse at 0900 h and the temperature of each soil recorded 6 h later. A mean soil temperature of 25.2°C was obtained for the oiled soil compared to 24.1°C for the unoiled control. Temperature 1 cm above the pots at the time of recording was 31.0°C.

The significance of this observation is discussed in Chapter VII.

(21) pH of Main Field Plots

pH measurements were made of selected soil plots to investigate possible pH changes for successive samplings and the effect on pH of oil application rate. Determinations were made for field samples (sampled as previously described p. 88) from plots 107, 224 (0 t/ha), 118, 202 (56 t/ha), 123, 222 (112 t/ha), 122, 211 (168 t/ha) and 108, 219 (224 t/ha) all of which represented tillage level 3 plots. Assuming continual aeration was important to the degradation of waste

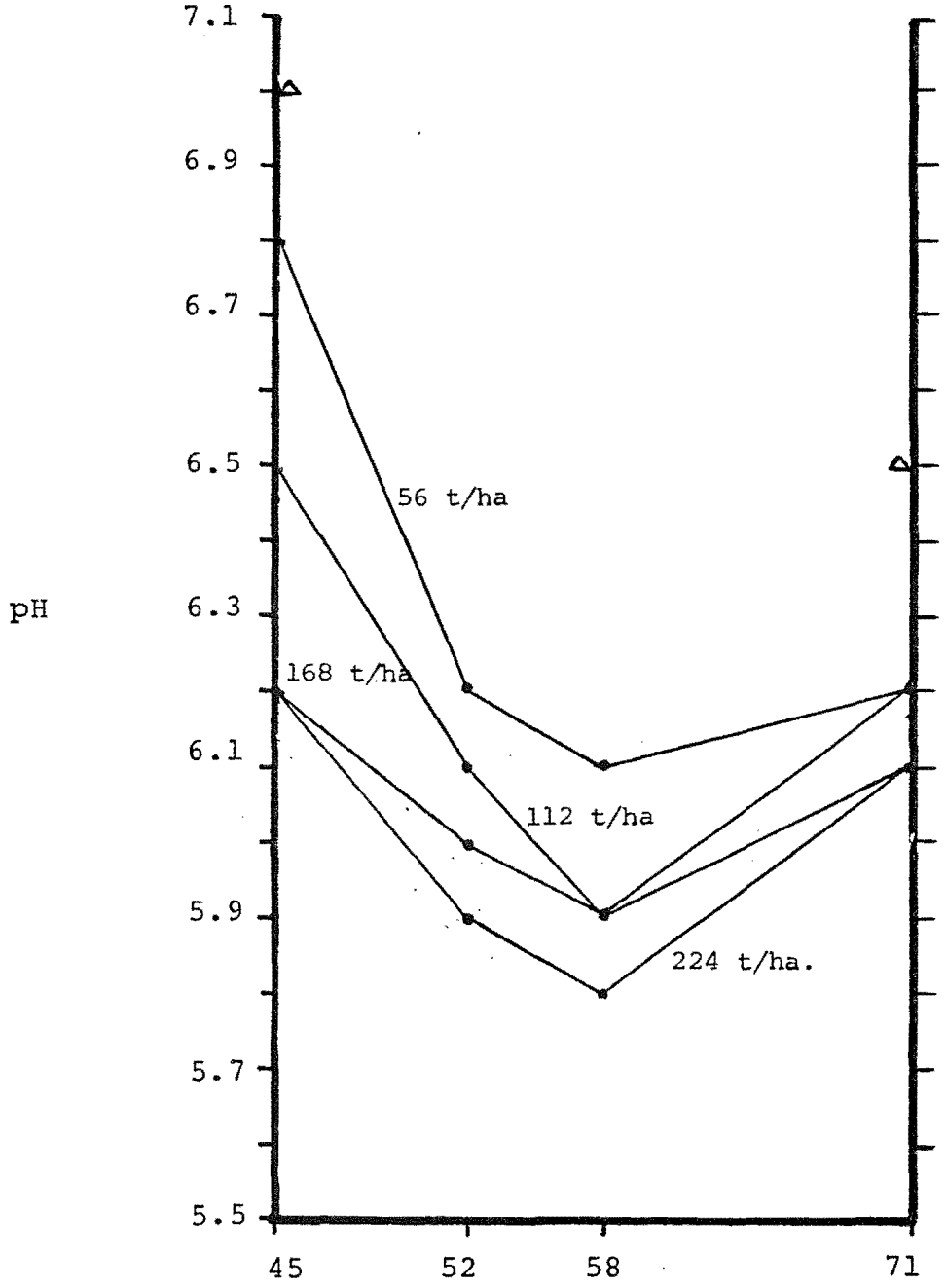
lubricating oil any pH changes might be greatest on these plots. Using the F.A.O. method (p. 67), three measurements were made on each sample (stored at 4°C) at the conclusion of the field trial. Measurements were made on samples taken 45, 52, and 71 weeks after oil application except for 0 t/ha plots for which 52 and 58 week samplings were omitted. Results shown in Table 23 indicated that 45 weeks after oil application (third sampling), pH of the Timaru field soil had been reduced by a maximum of 0.8 pH unit in the presence of waste lubricating oil. A decrease in pH occurred for oil application rates up to 168 t/ha. The addition of further oil to provide a 224 t/ha oil application rate had no effect. Total acid number of the waste lubricating oil was 4.5 mg KOH/g of oil as determined by the American Society for Testing and Materials (1975) method D664-IP177 (Shell Oil New Zealand Ltd. pers. comm. 1973a). Total acid number refers to the quantity of base, expressed in mg KOH that is required to neutralize all acidic constituents present in 1 g of sample. The pH of all oiled plots reached a minimum, 58 weeks after oil application but an increase was recorded for 56, 112, 168 and 224 t/ha oil application rates 13 weeks later (sixth sampling). Although the unoiled control plot pH decreased from 45 to 71 weeks after oil application, it remained higher than that obtained for plots of the lowest oil application rate. The decrease in pH of the oiled soils may have been due to the acidity of the oil but was not inconsistent with the increased production of organic acids on oiled soils observed by Kincannon et al. (1972).

Table 23: pH of Timaru main field plots after oil application.
† Means of 6 samples from east and west block plots; soil: water 1:2.5.

	pH†			
	Weeks after oil application			
	45	52	58	71
Oil Application Rate (t/ha)				
0	7.0	-	-	6.5
56	6.8	6.2	6.1	6.2
112	6.5	6.1	5.9	6.1
168	6.2	6.0	5.9	6.2
224	6.2	5.9	5.8	6.1

Figure 13: Effect of oil application rate and time on pH of Timaru silt loam. pH was not recorded until 45 weeks after oil application because of earlier difficulties with the soil sampling method.

△ Unoiled control. Soil:water 1:2.5.



(22) Micro-organisms from Main Field Plots

ZoBell (1946) reviewed criteria for use by micro-organisms of liquid and solid hydrocarbons. He considered use to be the chemical modification of hydrocarbons with or without loss of carbon atoms from the molecule. Of these he suggested the most common to be their modification by micro-organisms in liquid media. Emulsification by micro-organisms of various kinds of oil and resulting colour changes have been observed and the complete disappearance of the oil is not uncommon. Extensive multiplication of micro-organisms(as determined by cell counts after a few days) in mineral salts solution enriched with gaseous liquid and solid hydrocarbons was considered to indicate that the hydrocarbon had been utilized.

Raymond et al. (1976) attempted to enumerate the hydrocarbon utilizing microflora present in soils to which a range of oils including waste crankcase oils, crude oils, fuel oil and heating oil had been added. He reported difficulty with direct plating techniques and liquid culture tube systems. Elective culture techniques which incorporated oil in or on the surface of agar plates did not indicate that microbial use had occurred. The presence of hydrocarbon utilizing micro-organisms in soil contaminated with waste lubricating oil was determined by inoculating petri dishes of mineral salts agar. A gaseous substrate was provided by hexadecane saturated filter discs placed in the petri-dish lids. As conceded by the authors this single compound substrate may have detected only a small

fraction of the micro-organisms using the far more complex oils. In another study, ZoBell (1946) reported microbial growth on the surface of inoculated agar over which light oils had been poured. Microbial growth was also observed in liquid cultures covered with a thin layer of liquid hydrocarbons. Thickness of the layer did not affect growth of the micro-organisms.

The aim of the present preliminary experiments was to enumerate the bacterial population in oiled and unoiled plots, to attempt identification of the most frequently isolated species and assess their ability to use hydrocarbons as a sole carbon source.

Eighteen months after oil application, soil samples were removed from Timaru field plots selected to represent 0.56 and 224 t/ha oil application rates. Three 100 ml soil samples were randomly sampled, returned to the laboratory and stored at 4°C until use 7 d later. To reduce contamination during sampling, cleaning jars and sample spoon were rinsed thoroughly with 90 percent ethanol. Samples were mixed with a glass rod and a 10^{-6} dilution of each soil sample made following the procedure of F.A.O. Soils Bulletin no. 7 (1967). Solution (0.1 ml) was plated out in triplicate onto Bushnell and Haass (1941) mineral salts solution ($MgSO_4.2H_2O$ 20g, $CaCl_2$ 2.0g, KH_2PO_4 100 g, K_2HPO_4 100 g, NH_4NO_3 100 g, $FeCl_3$ 100 drops concentrated soln/ℓ) solidified with 1.0 percent agar (Oxoid no.1) autoclaved at 103.5 kPa. The pH was adjusted to 6.8 using 0.1N NaOH. Approximately 0.1 ml of sterile (0.45μ millipore filtered) 'Vitrea 22'[+] oil.

[†] 'Vitrea 22' lubricating oil was used because it is pure paraffinic hydrocarbon of similar molecular weight range to the largely paraffinic waste lubricating oil.

(Shell Oil New Zealand Ltd.) was spread aseptically over all plates. Three uninoculated control plates were similarly prepared. All plates were incubated at 25°C for 4 d and inspected for microbial growth. Mean colony counts of 8.2×10^6, 3.1×10^6 and 5.0×10^3/ml were obtained for 224, 56 and 0 t/ha oil application rates respectively. Bacteria were the only micro-organisms observed on the plates and no colonies were observed on the control plates. A quantitative comparison between treatment and control plot populations is unlikely to be valid because of differences in moisture content but significantly greater numbers were recorded from oil treated soil. Two morphovars, A1 and B1 dominated the plates with lesser numbers of few others. These were tentatively identified from their morphological characteristics and standard biochemical tests (Appendix 2) as belonging to genera *Achromobacter* and *Proteus*.

A further experiment was performed to investigate the modification of 'Vitrea' oil by the *Achromobacter* isolate. Nine 15 x 2.3 cm boiling tubes each containing 30 ml of Bushnell and Haass (1941) mineral salts solution were stoppered with cotton wool bungs and autoclaved. Approximately 0.5 ml of 0.45µ millipore filtered 'Vitrea' oil was added aseptically to each of three loosely stoppered boiling tubes which had been inoculated with *Achromobacter* isolate. Three inoculated tubes containing mineral salts solution served as controls. All tubes were shaken daily and returned to the incubator. A turbid solution in the inoculated tubes overlain with oil was observed after 3 d.

Small droplets of oil in suspension throughout the tubes appeared responsible for the effect. After 7 d the undersurface of the oil in contact with the medium appeared yellowish white in colour and ropy. Upon shaking, parts of the surface broke away and remained in suspension. No changes were observed for any of the control tubes. Similar effects reported by other workers have been reviewed by ZoBell (1946). They attributed the microbial emulsification of oil to the production of organic acids, surface active agents and carbon dioxide and in part to the penetration of the oil by micro-organisms.

After 8 d incubation the tubes were shaken and samples taken for haemocytometer counts. Large numbers (uncountable) of bacteria were present in the inoculated oiled solution, few in the unoiled inoculated solution and none in the unoiled uninoculated control. On this evidence it was concluded that the isolated *Achromobacter* species was able to use hydrocarbons for growth when supplied as a sole carbon source.

CHAPTER III

VOLATILES PRODUCED BY OILED TIMARU SOIL

In the course of sampling the Timaru field trial it was noticed that several plots smelled strongly of oil. Since some oil might be lost by evaporation an alternative, gas chromatographic method for checking oil disappearance could be developed providing the concentration of volatiles was simply related to soil oil concentration. An experiment was conducted to determine whether volatiles could be detected from soils containing a range of oil concentrations.

I. METHODS

Air dried and 4 mm sieved soil from the third sampling (see Chapter II) of field plots as shown in Table 24 was used for the investigation. Soil from an additional plot of the 0 and 56 t/ha oil application rates was included to determine whether volatile production was being affected by soil organic matter content. Eighty g of soil from plot 200 was weighed into each of three 550 ml, 9.0 cm i.d. 'Agee' screw type jars. A 1.0 cm hole was drilled in the lid of each jar before fitting it with a rubber septum. Each jar was flushed with air and sealed by the lid held in place by a screw type metal ring. All samples were similarly prepared. Three further jars each containing

Table 24: Timaru field plots investigated.

Plot no.	Field oil application rate	Oil concentration*†1	Organic*†2 matter content	moisture*†3
200	0	N.D.	0.102	0.355
224	0	N.D.	0.192	0.413
203	56	0.066	0.118	0.440
221	56	0.064	0.193	0.512
206	112	0.139	0.115	0.485
204	168	0.179	0.113	0.443
212	224	0.352	0.156	0.418

* means of 6 replicates

† g/5g air-dried oiled soil.

1. Cold extractable oil corrected for 100 percent recovery.
2. Weight loss of extracted soil upon ignition (380°C 16 h). Corrected for 100 percent oil recovery.
3. Weight loss of O.D. (105°C) unextracted air-dried soil.

only 80 g of oil provided the control. All jars were incubated at 25°C for 26 d and on day 27 they were removed for gas chromatography. Each jar was shaken vigorously immediately before a 2 ml plastic disposable syringe was used to sample the atmosphere above the soil. Samples were injected into a 'Tracor' 550 Gas Chromatograph with a flame ionization detector. A 1.22 m x 3.2 mm copper column packed with 100 mesh deactivated alumina (Smith and Dowdell 1973) was used for the experiment with a nitrogen flow of 60 ml min^{-1} and oven temperature of 90°C. Detector and injection ports were at 270°C and 120°C respectively. Ethylene and acetylene standards were dilutions of commercial gas (Matheson's Ltd. Box 188 Newark, California 94560 U.S.A.).

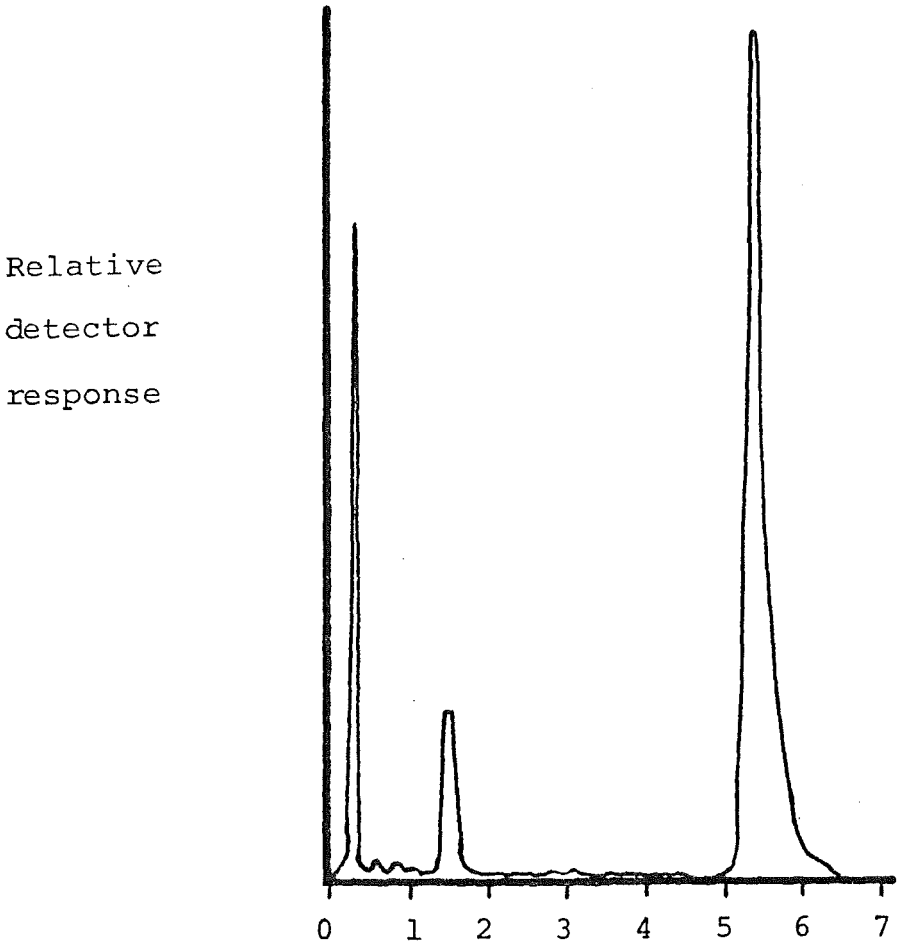
II. RESULTS

Three prominent peaks as shown in Figure 15 were obtained for all soils. Comparison of peak retention times with those of ethylene, ethane, propane, pentane and hexane suggested a low carbon number for the peaks the first of which may be ethylene (Figure 16).

The data were analysed by analysis of variance. A 2-way analysis was used to separately treat the effect of organic matter. Results are presented in Tables 25 and 26.

These results indicatedthat presence of oil in soil had stimulated the production of gases. No volatiles

Figure 14 : Gas chromatography of volatiles from field plot 219. Oil application rate 224 t/ha (sampling 1.).



(Chart recorder speed 1 cm/min)

Figure 15: Gas chromatography of standards.

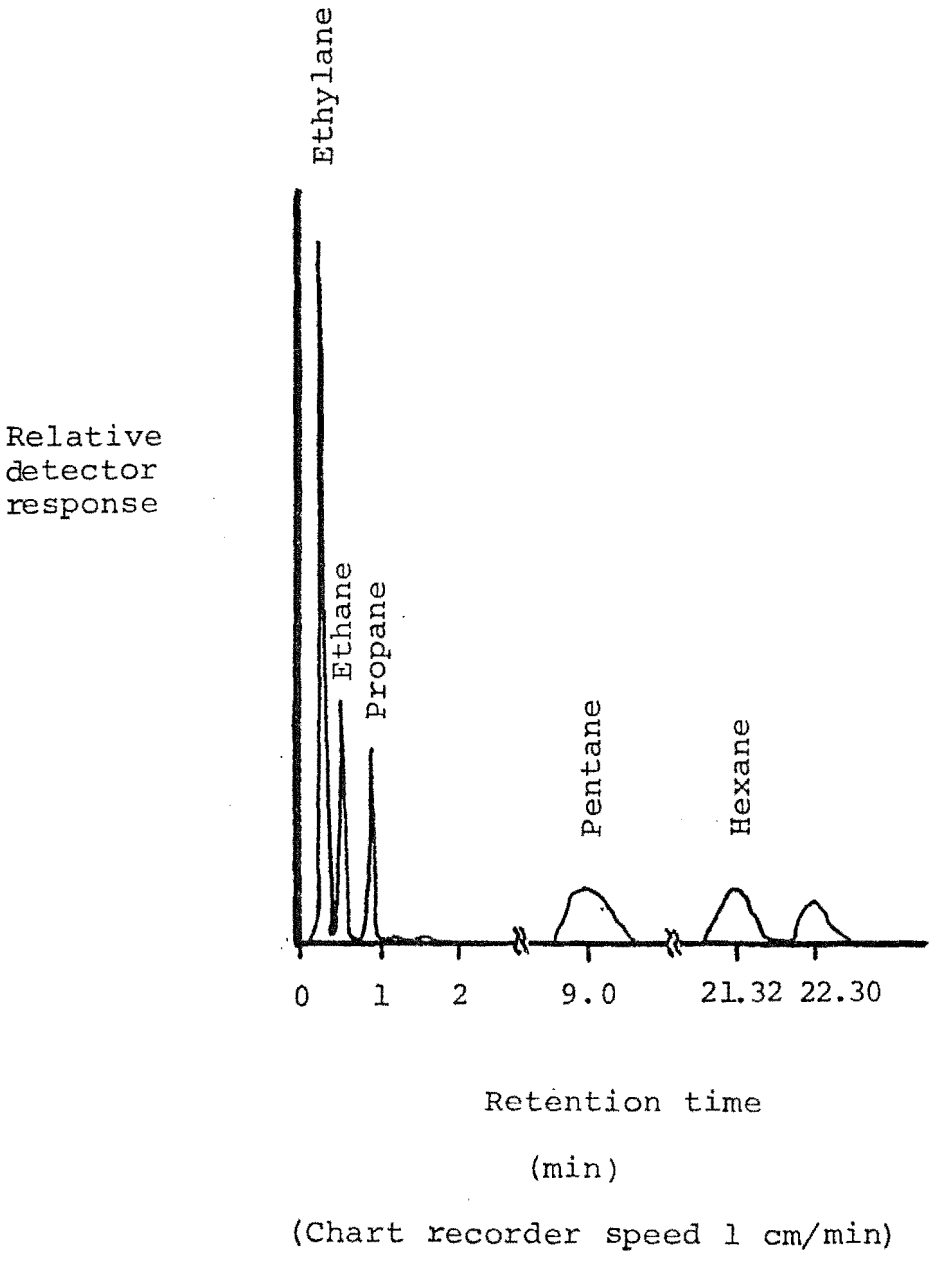


Table 25: Volatile production by oiled Timaru silt loam after 27 days.
† Means of three samples

	Plot no. 200	203	206	204	212	Control	Ethylene
Oil application rate (t/ha)	0	56	112	168	224	oil only	1ppm
Relative log peak height 0.15†	1.91	1.78	2.01	1.67	1.74	N.D.	1.64
	S.E. = ± 0.030800 L.S.D. (5%) = 0.10 F = 10.92***						
Relative log peak height 1.35†	0.33	0.78	1.08	1.02	1.09	N.D.	1.64
	S.E. = ± 0.022900 L.S.D. (5%) = 0.07 F = 118.61***						
Relative log peak height 5.35†	0.11	0.72	1.25	1.32	0.96	N.D.	1.64
	S.E. = ± 0.040000 L.S.D. (5%) = 0.13 F = 148.87***						

Table 26 : Effect of soil organic matter on volatile production by oiled Timaru silt loam after 27 days.

a(i) Main effect-oil*. Production of volatile 0.15.

† Means of 6 samples.

Soil plot	Oil application rate (t/ha)	Mean relative log peak height†
200 & 224	0	1.91
203 & 221	56	1.99

S.E. = ± 0.020615 L.S.D. 5% = 0.07

a(ii) Main effect-organic matter***. Production of volatile 0.15. † Means of 6 samples.

Soil plot	Soil organic matter	Mean relative log peak height†
200 & 203	Low	2.07
224 & 221	High	2.38

S.E. = ± 0.020615 L.S.D. (5%) = 0.07

a(iii) Interaction-oil x organic matter***. Production of volatile 0.15. † Means of 3 samples.

	Mean relative log peak height †	
	Unoiled	Oiled
Low soil organic matter	1.91 (200)	1.78 (203)
High soil organic matter	1.92 (224)	2.19 (221)

S.E. = ± 0.029154 L.S.D. (5%) = 0.10

Soil plot no. in parentheses.

b(i) Main effect - oil***. Production of volatile 1.35. † Means of 6 samples

Soil plot	Oil application rate (t/ha)	Mean relative log peak height†
200 & 224	0	0.28
203 & 221	56	0.54

S.E. = ± 0.113130 L.S.D. (5%) = 0.04

b(ii) Main effect-organic matter***. Production of volatile 1.35. † Means of 6 samples.

Soil plot	Soil organic matter	Mean relative log peak height†
200 & 203	Low	0.56
224 & 221	High	0.26

S.E. = ± 0.113130 L.S.D. (5%) = 0.04

b(iii) Interaction - oil x organic matter***.

Production of volatile 1.35. † Means of 3 samples.

	Mean relative log peak height †	
	Unoiled	Oiled
Low soil organic matter	0.33 (200)	0.78 (203)
High soil organic matter	0.22 (224)	0.30 (221)

S.E. = ± 0.160000 L.S.D. (5%) = 0.06

Soil plot no. in parentheses.

c(i) Main effect - oil***. Production of volatile 5.35. † Mean of 6 samples.

Soil plot	Oil application rate (t/ha)	Mean relative log peak height†
200 & 224	0	0.12
203 & 221	56	0.81

S.E. = ± 0.226710 L.S.D. 5% = 0.08

c(ii) Main effect - organic matter***. Production of volatile 5.35. † Mean of 6 samples.

Soil plot	Soil organic matter	Mean relative log peak height†
200 & 203	Low	0.42
224 & 221	High	0.51

S.E. = ± 0.226710 L.S.D. (5%) = 0.08

c(iii) Interaction - oil x organic matter* Production of volatile 5.35. † means of 3 samples.

	Mean relative log peak height†	
	Unoiled	Oiled
Low soil organic matter	0.11 (200)	0.72 (203)
High soil organic matter	0.13 (224)	0.89 (221)

S.E. = ± 0.032062 L.S.D. (5%) = 0.11

Soil plot no. in parentheses.

were detected from the jars containing oil alone and no reports could be found of volatile hydrocarbons being produced from the microbial decomposition of oil. Volatile concentration did not relate simply to oil concentration and was increased for oiled soils having a greater organic matter content. There appeared to be an optimum oil concentration for corresponding volatile levels, (112 and 168 t/ha oil application rates for peaks 0.15 and 5.35 respectively).

Soils which produced larger concentrations of volatiles also had higher soil moisture contents (Table 24). The observed stimulation in volatile production may therefore have been related to the additional soil moisture. To test this possibility, distilled water was injected into one 200 replicate jar to raise the soil moisture content to that of soil 224. All jars were shaken vigorously and incubated again. Two further samplings were made on days 37 and 55.

Results were analysed as before and are presented in Tables 27, 28,29 and 30 and Figures 17, 18 and 19.

With the exception of peaks 0.15 sampling 1, the presence of oil stimulated volatile production for all samplings. An initial oil application rate of 112t/ha and oil concentration of 2.8 percent at the time of the experiment appears to be optimum for the treated soils investigated.

The increased moisture content of the 200 replicate did not affect the response of the second and third

Table 27: Volatile production by oiled Timaru silt loam after 37 days.

† Means of 3 samples.

	Plot no.						
	200	203	206	204	212	Control	Ethylene
Oil application rate (t/ha)	0	56	112	168	224	oil only	1ppm
Relative log peak height† 0.15	2.00	2.15	2.55	2.21	2.22	N.D.	1.64
	S.E. = ± 0.020600 L.S.D. (5%) = 0.07 F = 57.01***						
Relative log peak height†	0.76	1.26	1.50	1.43	1.44	N.D.	1.64
	S.E. = ± 0.022800 L.S.D. (5%) = 0.07 F = 106.52***						
Relative log peak height† 5.35	0.64	1.20	1.73	1.62	1.35	N.D.	1.64
	S.E. = ± 0.025200 L.S.D. (5%) = 0.08 F = 172.91***						

Table 28: Effect of soil organic matter on volatile production by oiled Timaru silt loam after 37 days

a(i) Main effect - oil***. Production of volatile 0.15

† Means of 6 samples.

Soil plot	Oil application rate (t/ha)	Mean relative log peak height†
200 & 224	0	2.04
203 & 221	56	2.41

S.E. = ± 0.025159 L.S.D. (5%) = 0.09

a(ii) Main effect - organic matter***. Production of volatile 0.15. †Means of 6 samples.

Soil plot	Soil organic matter	Mean relative log peak height†
200 & 203	Low	2.07
224 & 221	High	2.38

S.E. = ± 0.025159 L.S.D. (5%) = 0.09

a(iii) Interaction - oil x organic matter***.

Production of volatile 0.15. † Means of 3 samples

	Mean relative log peak height†	
	Unoiled	Oiled
Low soil organic matter	2.00 (200)	2.15 (203)
High soil organic matter	2.08 (224)	2.67 (221)

S.E. = ± 0.035894 L.S.D. (5%) = 0.12

b(i) Main effect - oil***. Production of volatile 1·35. † Means of 6 samples.

Soil plot	Oil application rate (t/ha)	Mean relative log peak height†
200 * 224	0	0.74
203 & 221	56	1.06

S.E. = ± 0.031000 L.S.D. (5%) = 0.11

b(ii) Main effect - organic matter***. Production of volatile 1.35. †Means of 6 samples.

Soil plot	Soil organic matter	Mean relative log peak height†
200 & 203	Low	1.01
224 & 221	High	0.80

S.E. = ± 0.031000 L.S.D. (5%) = 0.11

b(iii) Interaction - oil x organic matter***. Production of volatile 1.35. † Means of 3 samples.

	Mean relative log peak height †	
	Unoiled	Oiled
Low soil organic matter	0.76 (200)	1.26 (203)
High soil organic matter	0.74 (224)	0.86 (221)

S.E. = ± 0.043840 L.S.D. (5%) = 0.15

c(i) Main effect - oil***. Production of volatile 5.35. † Means of 6 samples.

Soil plot	Oil application rate (t/ha)	Mean relative log peak height†
200 & 224	0	0.61
203 & 221	56	1.36

S.E. = ± 0.002172 L.S.D. (5%) = 0.16

c(ii) Interaction - oil x organic matter. Production of volatile 5.35. † Means of 3 samples.

	Mean relative log peak height†	
	Unoiled	Oiled
Low soil organic matter	0.64 (200)	1.20 (203)
High soil organic matter	0.58 (224)	1.53 (221)

S.E. = ± 0.065916 L.S.D. (5%) = 0.23

Soil plot no. in parentheses.

Table 29: Volatile production by oiled Timaru silt loam after 55 days.

† Means of 3 samples.

	Plot no.						
	200	203	206	204	212	Control	Ethylene
Oil application rate (t/ha)	0	56	112	168	224	oil only	lppm
Relative log peak height† 0.15	2.19	2.37	2.50	2.27	2.07	N.D.	1.64
	S.E. = ± 0.039400 L.S.D. (5%) = 0.13 F = 9.32***						
Relative log peak height† 1.35	0.69	1.23	1.37	1.29	1.39	N.D.	1.64
	S.E. = ± 0.036100 L.S.D. (5%) = 0.12 F = 28.49***						
Relative log peak height† 5.35	0.73	1.61	1.58	1.39	1.28	N.D.	1.64
	S.E. = ± 0.052300 L.S.D. (5%) = 0.17 F = 21.09***						

Table 30: Effect of soil organic matter on volatile production by oiled Timaru silt loam after 55 days.

a(i) Main effect - oil***. Production of volatile 0.15. † Means of 6 samples.

Soil plot	Oil application rate (t/ha)	Mean relative log peak height†
200 & 224	0	2.15
203 & 221	56	2.49

S.E. = ± 0.035916 L.S.D. (5%) = 0.12

a(ii) Interaction-oil x organic matter***. Production of volatile 0.15. † Means of 3 samples

	Mean relative log peak height†	
	Unoiled	Oiled
Low soil organic matter	2.19 (200)	2.37 (203)
High soil organic matter	2.10 (224)	2.61 (221)

S.E. = ± 0.050882 L.S.D. (5%) = 0.18

Plot no. in parentheses.

b(i) Main effect - oil***. Production of volatile 1.35. † Means of 6 samples.

Soil plot	Oil application rate (t/ha)	Mean relative log peak height†
200 & 224	0	0.69
203 & 221	56	1.11

S.E. = ± 0.025980 L.S.D. (5%) = 0.09

b(ii) Main effect - organic matter**. Production of volatile 1.35. †Means of 6 samples.

Soil plot	Oil application rate (t/ha)	Mean relative log log peak height†
200 & 203	0	0.96
224 & 221	56	0.84

S.E. ± 0.025980 L.S.D. (5%) = 0.09

b(iii) Interaction - oil x organic matter**.

Production of volatile 1.35. † Means of 3 samples

	Mean relative log peak height†	
	Unoiled	Oiled
Low soil organic matter	0.69 (200)	1.23 (203)
High soil organic matter	0.69 (224)	0.99 (221)

S.E. = ± 0.036742 L.S.D. (5%) = 0.13

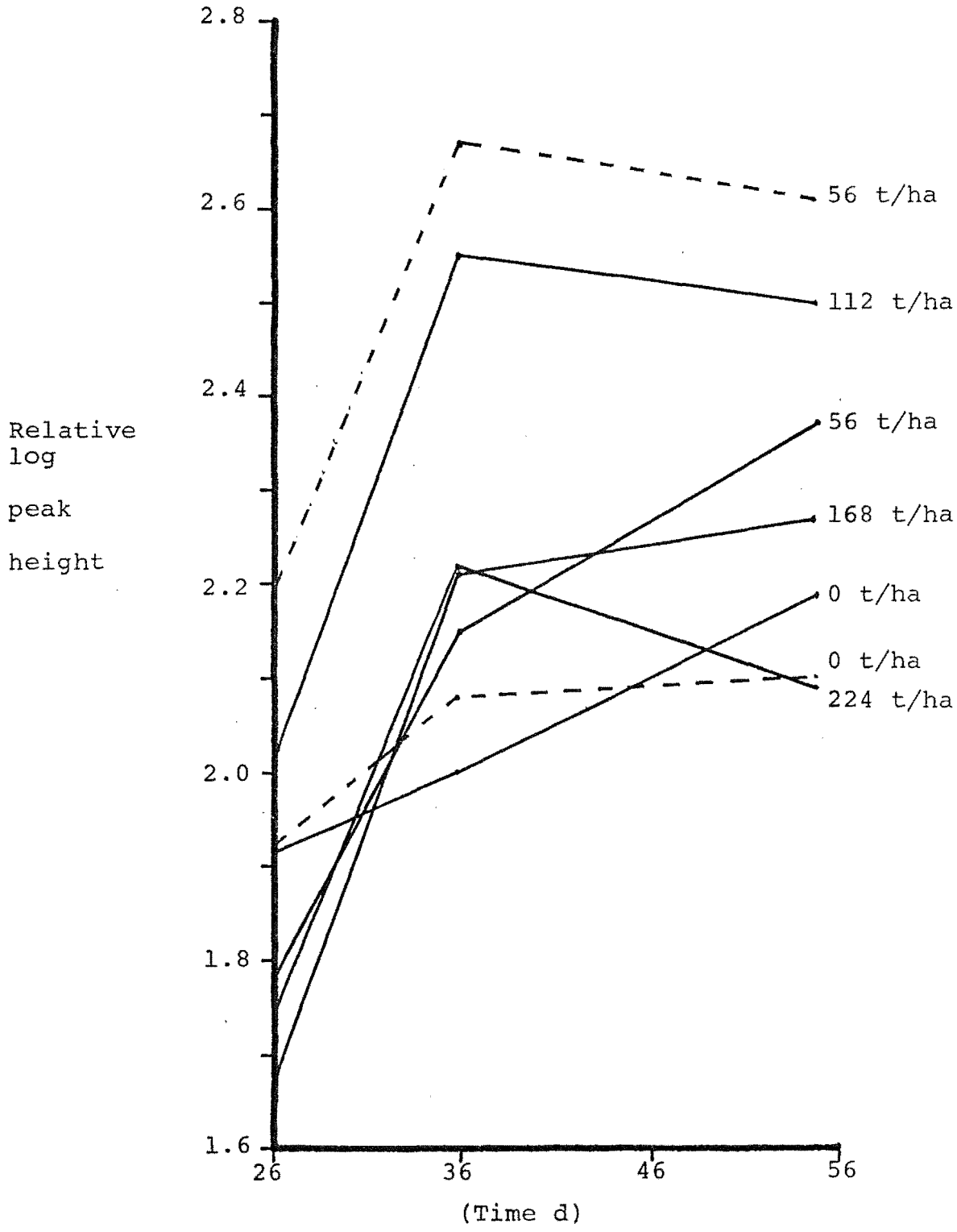
c(i) Main effect - oil***. Production of volatile 5.35. † Means of 6 samples.

Soil plot	Oil application rate (t/ha)	Mean relative log peak height†
200 & 224	0 (200)	0.71 (203)
203 & 221	56 (224)	1.59 (221)

S.E. = ± 0.023748 L.S.D. (5%) = 0.08

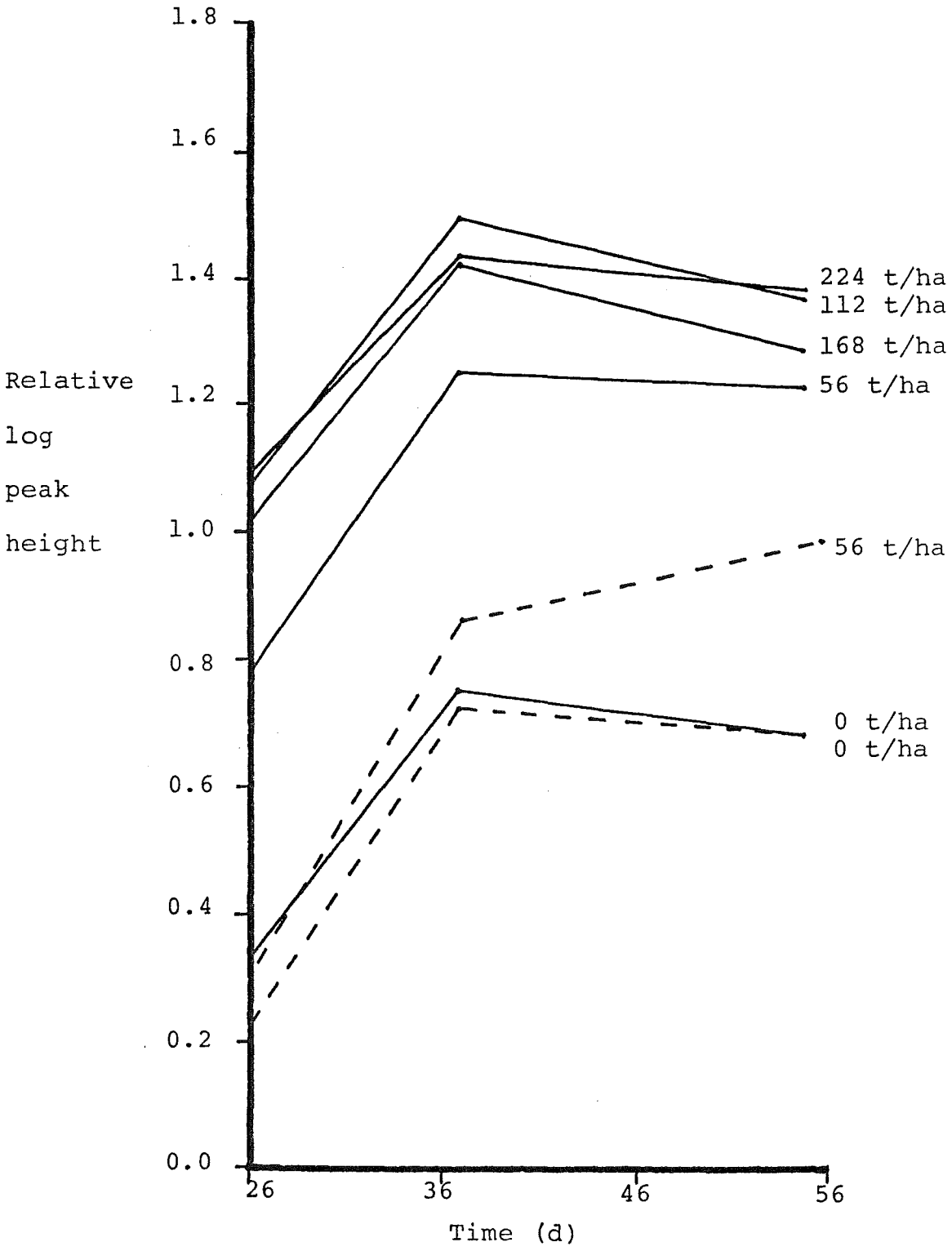
Soil plot no. in parentheses.

Figure 16: Volatile production from Timaru oiled silt loam (peak 0.15)



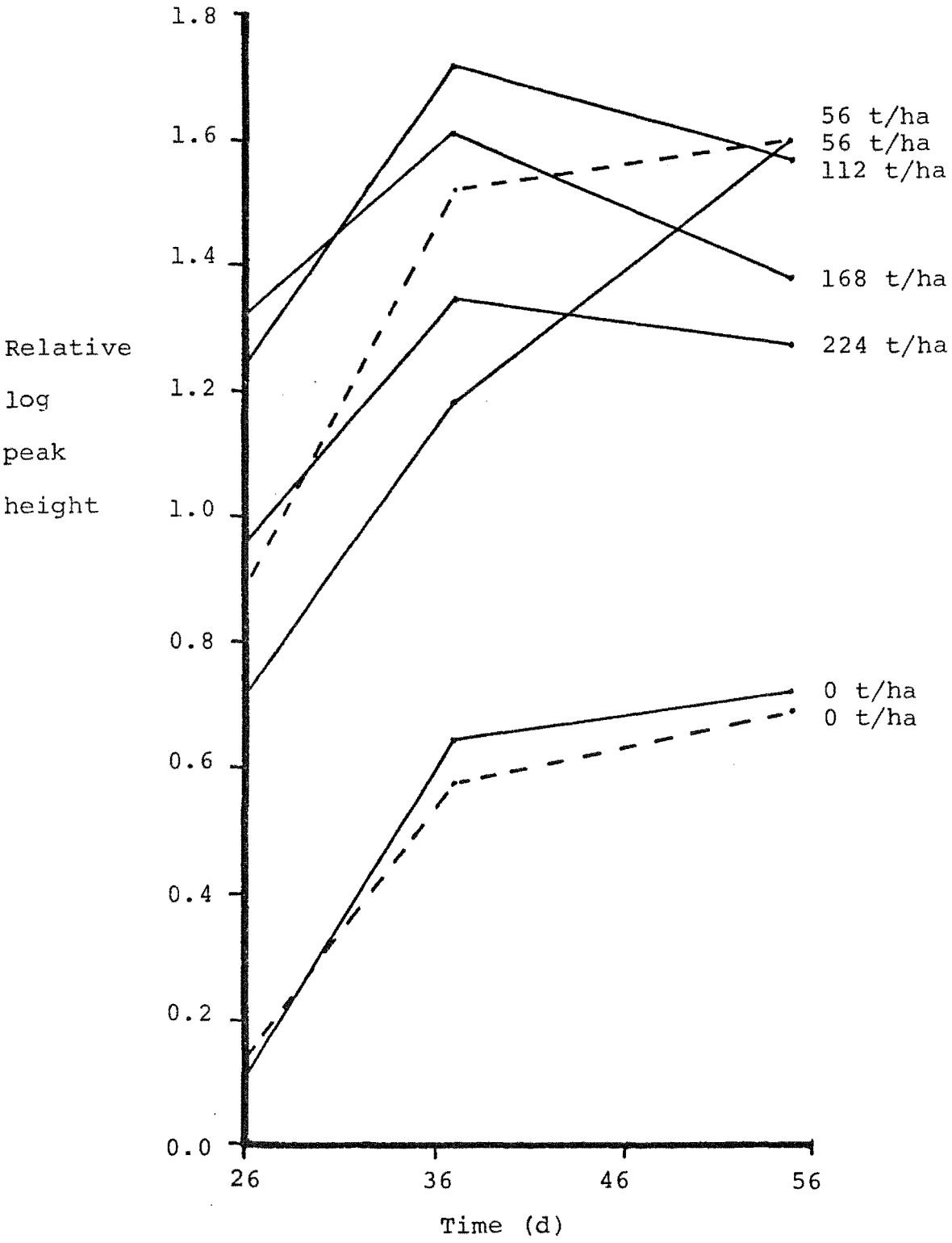
Broken line denotes plots having a higher soil organic matter content

Figure 17: Volatile production from Timaru oiled silt loam (peak 1.35)



Broken line denotes plots having a higher soil organic matter content

Figure 18: Volatile production from Timaru oiled silt loam (peak 5.35)



Broken line denotes plots having a higher soil organic matter content.

samplings thereby suggesting that moisture is not directly responsible for the stimulation. Production from all oiled soils except that of 56 t/ha decreased after the second sampling, the concentration from the latter remaining almost constant (1.35) or increasing (0.15 and 5.35).

Peak response from the 56 t/ha high organic matter soil was consistently greater for peaks 0.15 and 5.35 than for its lower organic matter equivalent compared to those from the 0 t/ha soils which were generally similar. Volatile concentration from most of the oiled soils decreased after the second sampling.

Results indicated that low molecular weight volatiles were produced by both unoiled soils and soils to which waste lubricating oil had been added. No volatiles were produced by the oil only control. Larger 0.15, 1.35 and 5.35 peak heightswere obtained for all oiled soils compared to those of unoiled soil. Higher soil organic matter content did not consistently increase peak height except for 56 t/ha oil application rate peak 0.15. The same retention times obtained from each peak on unoiled and oiled soils suggested that the presence of oil had directly or indirectly stimulated production of the same compounds. Conceivably the volatiles could have been produced by biochemical or chemical activity and caution should therefore be exercised when interpreting experiments in which CO_2 evolution or O_2 uptake is used to measure to decomposition rate of an organic compound added to soil.

Complexity of the results precluded the use of volatile production for following the loss of waste lubricating oil from soil.

CHAPTER IV

THE EFFECT OF WASTE LUBRICATING OIL ON PLANT GROWTH

Land is an important food producing resource and should, after its use for the disposal of waste lubricating oil, be left in a state which is not permanently toxic to plants.

Few investigations have been made into the effects of oil on seed germination and plant growth. Schwendinger (1968) found reduced dry matter and germination of various plant species in pot experiments containing soils to which crude oil (not waste oil) had been added. No reasons were advanced for the observed effects. Similar work by Plice (1948) under field conditions showed that such effects may diminish with time and four years after oil application larger plant yields than the controls were obtained. The stimulation was sustained for a further crop after which the experiment was concluded.

Field observations of sward height and density at the Timaru oil disposal site 72 weeks after oil application suggested that growth of self sown *Agrostis tenuis* and *Poa pratensis* on uncultivated plots of all oil application rates had not been reduced compared to that of the unoiled uncultivated control plots. Experiments were conducted to investigate the effects of oil residues from cultivated plots on seed germination and plant growth. In this thesis, the term oily residues refers

to hexane extractable oil compounds which include any products of alteration.

I. THE EFFECTS OF WASTE LUBRICATING OIL ON SEED GERMINATION AND SEEDLING EMERGENCE OF *Lolium perenne* L. "GRASSLANDS RUANUI"

(1) Methods

The soils used were taken from the Timaru site 72 weeks after oil application and represented tillage level 3 for all application rates. Air-dried soil from each of plots 224 (0 t/ha), 118 (56 t/ha), 123 (112 t/ha), 122 (168 t/ha), and 219 (224 t/ha) was sieved (4 mm) and three 1 ℓ plastic pots filled with about 1 kg of each soil were brought to near water holding capacity with distilled water. Similar plastic pots immersed in waste lubricating oil for 24 h were not physically altered suggesting them to be inert. The oil concentration of the soils was determined by Soxhlet extraction (see p. 62).

Lolium perenne L. "Grasslands Ruanui" (perennial ryegrass) was selected for the bioassay experiments because it had previously been shown to take up lead into its shoot system. This was an important requirement because one aspect of the study was to investigate the uptake of lead residues by plants from the land disposal system. Five ryegrass seeds were planted 0.5 cm below the soil surface and covered with fresh soil. The seed had previously been surface sterilized in 3 percent hypochlorite solution

to reduce the chances of infection by spores present on the seed. Preliminary experiments had shown that the germination rate of the ryegrass seeds on moist filter pads was not affected by hypochlorite pre-treatment. All pots were incubated at 25°C (16 hd) for 21 d after which the percentage seedling emerged (percentage of those planted) was determined for each pot. Equal amounts of distilled water were maintained to the pots as required and never exceeded the water holding capacity of the soils.

(2) Results

Results for seedling emergence as shown in Table 31 were analysed using a one way analysis of variance.

Table 31: Seedling emergence and oil concentration for Timaru field experiment soils. * Means of 3 replicates. † Means of 5 replicates.

	Initial oil application rate (t/ha)				
	0	56	112	168	224
Mean oil concentration g/5g air dried soil*	N.D.	0.06	0.10	0.13	0.43
Mean no. seedlings after 21 d †	3.50	4.30	5.00	3.33	3.33

F = 2.04 n.s.

The non significant F value indicated that the presence of oil residues at these concentrations had no significant effect on seedling emergence. Microscopal examination of the ungerminated seeds showed none to have been visibly altered. Results of Schwendinger (1968) were of dubious value because the experiments were not replicated and no analysis of his results was given. Comparison with those of the present study was therefore inappropriate. Waste lubricating oil was initially applied to the Timaru soil at a maximum rate of 224 t/ha or 12 percent w/w, a concentration at which no germination was obtained. As indicated by the results of the above experiment the reduced hexane extractable oil concentration (0.45 g/5g air dried soil or about 2.8 percent) obtained for the soil 72 weeks after oil application permitted normal germination showing that it was not toxic as judged by seedling emergence.

II THE EFFECT OF WASTE LUBRICATING OIL ON THE GROWTH OF *Lolium perenne* L. "GRASSLANDS RUANUI".

(1) Methods

The effect of waste oil on plant growth was examined to investigate the effect on plant growth of oily residues from the Timaru site. Seventy-two weeks after the study was begun, 20 kg soil samples were taken from each of plots 102 (0 t/ha) and 109 (224 t/ha). Adverse effects on crop yield have been shown to increase with increased

oil concentration of the soil (Plice 1948). From this observation it appeared justifiable to assume that the 224 t/ha field soil with a hexane extractable oil concentration of 1.9 percent was most likely to affect plant growth. Three 1d old Ruanui ryegrass seedlings germinated on filter pads for 3d at 20°C were planted in pots (3 replicates/soil) prepared as earlier described. Treatment and control pots were separately placed in two large petri dishes (3 pots/dish) and placed in a growth cabinet at 25°C (16 h d; light source fluorescent tubes and tungsten filament bulbs). The pots were bottom watered with tap water which was added to the petri dishes as required.

(2) Results

After 14 d, the control (102, 0 t/ha) plants by visual inspection were adjudged normal and had three leaves. Many of the treatment plants (109, 224 t/ha) by comparison showed chlorosis of the first leaf and stems were considerably smaller (width and length) compared with those of the control. These differences, similar in extent, were present on day 43 when the plants from each pot were removed along with the soil and placed in a 4 mm sieve. Soil adhering to the roots was washed through the sieve and the plants then separated into root and shoot. Root and shoot were oven dried (105°C) for 24 h and weighed. Results are recorded in Table 32.

Table 32: Yield of Ruanui ryegrass grown on oiled Timaru silt loam (weight in g).

	Control soil plot 102 (0 t/ha)	Treated soil plot 109 (224 t/ha)	Treatment as percentage of control	F
Whole plant	0.51	0.22	42.0	131.9***
Shoot	0.36	0.13	36.4	330.4***
Root	0.15	0.08	55.6	14.4***

C.V. = 1.2 percent

The results demonstrate that the weights of treatment whole plants, roots and shoots were significantly lower when compared with the control. The 64 percent reduction of shoot yield was much greater than the zero reduction for wheat, barley and rye plants grown 24 weeks after crude oil application to soil at 1 percent w/w obtained by Plice (1948). It is difficult to proffer an explanation for these results. Lead which occurs in waste lubricating oil at relatively high concentrations (see p.349), oil residues or both may have been directly or indirectly responsible. The higher root/shoot ratio obtained for the treatment plants suggested that stresses in the oiled soil may have induced morphological plant reaction. It was not possible in the time remaining to ascertain the

fact or processes responsible for this effect but it may be as observed by Johannson (1962) for soil contaminated with crude oil, that the time necessary for the restoration of normal plant growth could be reduced by the addition of nitrogenous fertilizers to the oiled soil. Poor soil aeration has been suggested by Schwendinger (1968) as responsible for reduced plant yield from oily soils.

III THE PERCENTAGE AND NATURE OF NITROGEN IN WASTE LUBRICATING OIL

Macronutrients (nitrogen, phosphorus and potassium) have been considered the nutrients most frequently limiting plant growth on New Zealand soils (During 1967). When large amounts of carbonaceous material are added to soil to give a high C:N ratio, large amounts of inorganic nitrogen are immobilized by bacteria (Russell 1961). Such a ratio could be expected at Timaru where waste lubricating oil had been added at rates of up to 224 t/ha. There did not appear to be any report in the literature concerning the amounts and nature of nitrogenous substances in waste lubricating oils but Davis (1967) stated that very small amounts of nitrogen containing compounds are present in crude oils as "... nitrogen bases, alkyl derivatives of quinoline and pyridine with a few non-aromatic bases" and porphyrins. The percentage and nature of nitrogen

occurring in a 4 ℓ sample of waste lubricating oil from the supply used for the Timaru field experiment, was investigated in the following experiments.

(1a) Methods

Total nitrogen in the oil was determined by the usual Kjeldahl method using a mercury catalyst followed by steam distillation of the ammonia in digests into boric acid back titrated with boric acid (Bremner 1965a). Inorganic nitrogen was determined by KCl extraction followed by steam distillation of aliquots with MgO for ammonia and Devardas alloy for nitrate and nitrate. The procedures for determining nitrogen were those given by Bremner (1965b). The nitrogen content of reagent blanks was determined and subtracted from that obtained for the oil.

(1b) Results

The results shown in Table 33 and 34 indicated that of the nitrogen applied to the field plots, a considerable amount was in organic combination.

Table 33: Nitrogen present in waste lubricating oil. Means of 3 samples

	Form		
	NH_3	NO_3,NO_2	Organic
Percentage N by weight	0.7	3.0	96.3
C.V.	0.0	20.2	0.0

Percent total nitrogen in waste lubricating oil = 0.108.

Table 34: Nitrogen applied to field plots as a component of waste lubricating oil.

Waste lubricating oil application rate (t/ha)	Nitrogen - rate of application kg/ha			
	NH_3	NO_3,NO_2	Organic N	Total N
56	0.4	1.8	58.3	60.5
112	0.8	3.7	116.5	121.0
168	1.2	5.5	174.8	181.5
224	1.6	7.4	233.0	242.0

(2a) Method

The nature of organic nitrogenous substances in waste lubricating oil was investigated by refluxing an amount of oil containing 10 mg N (Kjeldahl) with 6N HCl for 24 h and determining the nitrogen distribution of the hydrolysate (Bremner 1965c). Amino acid - N (α-amino-N) as ammonia and ammonia produced from other N containing organic compounds during hydrolysis were determined by Bremner's (1965c) method except that NaOH was used instead of phosphate-borate buffer. Non α-amino-N released as ammonia from a further 2.5 g sample of hydrolysate treated with 2.5 ml of 10N NaOH was determined by the method earlier described. Using the above method, nitrogen derived from α amino groups was also determined for unhydrolysed waste lubricating oil.

(2b) Results

Results given in Table 35 indicated that about 5 percent of the total nitrogen additional to the inorganic

Table 35: Organic nitrogen present in waste lubricating oil. (Method, Bremner 1965c).

Form of N occurring in hydrolysed and unhydrolysed waste lubricating oil	Weight $N\ ng\ g^{-1}$	Weight of nitrogen applied to soil for various oil application rates (kg N/ha) Oil application rate (t/ha)			
		56	112	168	224
α-amino + non α-amino acid nitrogen (hydrolysed waste lubricating oil)	54.8	3.1	6.1	9.2	12.3
non α-amino acid nitrogen (hydrolysed waste lubricating oil)	10.6	0.6	1.2	1.8	2.4
α-amino acid nitrogen (hydrolysed waste lubricating oil)	44.2	2.5	5.0	7.4	9.9
α-amino acid nitrogen (unhydrolysed waste lubricating oil)	9.9	0.6	1.1	1.7	2.2

or nitrogen present in the hydrolysate could be accounted for as α-amino-N. Twenty-two percent of the α-amino-N determined in acid hydrolysis could be determined directly in unhydrolysed

oil by the Bremner procedure. That the waste lubricating oil contained proteinaceous material is surprising because of 'cracking' during the production of lubricating oils and their working temperatures but proteinaceous material in the presence of large amounts of carbonaceous material has been shown to withstand ozonolysis at 550°C (Bremner private comm. 1973). Biological contamination of the waste lubricating oil may also have contributed to the result. There appeared to be no evidence to suggest that these proteinaceous compounds were unavailable for microbial growth since a large proportion of the nitrogen contained therein could be directly determined by the ninhydrin method. Most of the nitrogen in waste lubricating oil and other than proteinaceous material would appear to be in the form of complex nitrogenous compounds. Evidence from elsewhere indicated that the form of the nitrogen was likely to be heterocyclic and as such would not be estimated by ninhydrin or alkali distillation. Heterocyclic nitrogenous compounds such as pyridine derivatives exhibit varying degrees of resistance to microbial enzymes and were therefore unlikely to be readily available to the soil microflora.

Phosphorus was found to be present in the waste lubricating oil at a concentration of 0.046 percent by weight (Shell Oil New Zealand Ltd pers. comm. 1976). The phosphorus contribution from the oil and the superphosphate applied at 68 kg/ha (elemental P) to all plots was 68 (0 t/ha),

94 (56 t/ha), 120 (112 t/ha), 145 (168 t/ha) and 171 (224 t/ha) kg/ha. Phosphorus is added to lubricating oil as an organo-phosphate having a general formula of $(XO_3)PO$ where X = alkyl, aryl or alkyl-aryl group (Ford 1968). While the structure of the phosphorus compounds suggested that they could be readily degraded by soil microorganisms, some modification may have taken place during their life in internal combustion engines at relatively high temperatures. Consequently much of it may have been in a form unavailable to microorganisms and subsequently plants.

Minor amounts of potassium occurred in the waste lubricating oil (5 ppm Shell Oil Company pers. comm. 1976; Appendix 1), and contributions to the soil would have totalled 0.28 kg/ha (56 t/ha); 0.56 kg/ha (112 t/ha); 0.84 kg/ha (168 t/ha); and 1.42 kg/ha (225 t/ha).

Soil moisture retention capacity is an indication of the amounts of energy which must be expended by a plant to remove water from soil for its own requirements. Oily residues present in the soil may have affected its moisture retention capacity and so plant growth. Visual inspection of the structure of the oiled soils in the field suggested them to have been better aerated than the control.

IV THE EFFECTS OF WASTE LUBRICATING OIL ON (A) THE GROWTH OF *Lolium multiflorum* LAM. VAR. WESTERWOLDS "GRASSLANDS TAMA" AND (B) NUTRIENT STATUS AND MOISTURE RETENTION OF TIMARU SILT LOAM

Based on the above information and experimental results, further investigations were made to determine

(1) the effects on plant growth of oil residues in soils at all oil application rates

(2) whether normal plant growth (as determined by shoot dry matter yield) could be restored on the highest oil application rate soils

(3) to provide information on the availability of selected nutrients in oil treated soils

(4) to provide information on the soil moisture retention capacity of the oily soils.

(1) Effect of Waste Lubricating Oil on the Growth of *Lolium multiflorum* lam. var. Westerwolds "Grasslands Tama"

(a) Method

Eighty weeks after waste oil application about 15 kg of soil (uppermost 12 cm) was removed from each of the following Timaru field plots; 224 (0 t/ha), 118 (56 t/ha), 123 (112 t/ha), 122 (168 t/ha) and 219 (224 t/ha). All were tillage level three plots. Soxhlet extractable oil concentrations (g/5g air dried soil) and soil pH's were

respectively (0.000, 6.65), (0.060, 6.05), (0.103, 6.25), (0.130, 6.25) and (0.143, 6.00). Large pieces of vegetation were removed from the air-dried shredded soils each of which was used to fill 20 one ℓ plastic pots and each pot contained approximately 1 kg of soil. Solutions of nitrogen, phosphorus and potassium were prepared from NH_4NO_3, $CaHPO_4$ and KCl. The nutrient concentrations corresponded to application rates † of 224, 224 and 132 kg/ha respectively and were applied singly and in combination to provide a duplicated split plot design. The 50 ml nutrient solutions were mixed with the soil from the plots, replaced and air-dried for 4 d. An additional treatment was included to determine the effects of micronutrients on plant growth. Duplicate pots of each soil were amended with a combination of boron (1 kg/ha) as H_3BO_3, copper (5 kg/ha), as $CuSO_4.5H_2O$, iron (150 kg/ha) as $FeSO_4$, manganese (10 kg/ha) as $MnSO_4.4H_2O$, molybdenum (50 g/ha) as $Na_2MoO_4.2H_2O$ and zinc (10 kg/ha) as $ZnSO_4.7H_2O$.† Controls were provided for all soils. Soil micronutrient concentrations were unknown and because their addition may have raised concentrations to near toxic levels (which would mask the effects of the macronutrients), they were not added in combination with N, P and K. To obtain complete solution of the N, P and K, all solutions were made with distilled water and buffered to a pH of 2 with 0.1M HCl.

† Application rates were based on those commonly used in N.Z. farming practice (During, 1967: Fertilizers and Soils in New Zealand Farming).

Lolium multiflorum Lam. var. Westerwolds "Grasslands Tama" was used as a bioassay plant instead of *Lolium perenne* because it was a higher yielding tetraploid grass which required a shorter growing period. Greater genetic variability of the variety was a disadvantage and because of space restrictions it was necessary to reduce variation in plant yield. Seed weight was approximately linearly related to plant yield (K. Brown pers. comm. 1976) and therefore only seeds weighing between 4.0 and 5.0 mg were used. All pots recieved 100 ml of distilled water before the ryegrass seeds, surface sterilized as previously described were planted 6/pot. The pots were arranged in a growth cabinet (25^{o}C 16 h d) as a split-plot design. Pots within each treatment block were randomized.

Distilled water was added in equal amounts to the pots and the amount required which was determined visually, never exceeded the water holding capacity of the soils.

On day 33 the shoots were harvested, rinsed twice in distilled water and oven-dried (105^{o}C) for 24 h. The rinsing was necessary because the plants were later analysed for lead. Dry matter yield was determined to 0.005 g.

(b) Results (Crop 1)

On day 5 most of the pots had at least 3 emergent seedlings but some had 5 or 6. There was no obvious pattern of germination and all emerged seedlings were of similar size. After 8 d many seedlings had produced 2 leaves and

on day 11 (to further reduce plant variation), the pots were weeded to leave the three largest plants remaining in each pot. Removal of the smaller plants at this stage was justifiable because Tama ryegrass relies on endosperm for nutrients during its first two weeks of growth (K. Brown pers. comm.1976). On day 18 the plants on all soils were showing a response to nitrogen as indicated by leaf colour. Except in the unoiled control soil, leaves of plants growing on soils not amended with nitrogen were a yellowish green compared with the dark green of those on soils containing added nitrogen which presumably was available for plant uptake. Dry matter yields of the harvested shoots were recorded and analysed by factorial analysis of variance. Production of plants growing on micronutrient amended soils were analysed separately. Results are given in Tables 36 and 37.

The results for the first analysis, Table 36 show significant F values for main effects, nitrogen, phosphorus and oil and first order interactions, N x P, N x oil and P x oil. Mean yield for 168 t/ha and 224 t/ha soils was 90 percent of that obtained for 0, 56 and 112 t/ha soils. Similarly, reduced yields were obtained for 112, 168 and 224 t/ha soils to which a nitrogen amendment had not been made compared to those of 0 and 56 t/ha oiled nitrogen amended soils. The addition of nitrogen permitted the same or better yield on the 112, 168 and 224 t/ha soils. These results together with observations concerning nitrogen

Table 36 : Effect of nitrogen, phosphorus , potassium and oil on the shoot dry matter yield of Tama ryegrass grown on Timaru silt loam (crop 1). Analysis of variance table.

Source	d.f.	Mean square	F
Nitrogen	1	0.0739	142.66***
Phosphorus	1	0.0189	36.49***
Potassium	1	0.0006	1.16 n.s.
Oil	4	0.0051	9.85***
Nitrogen x phosphorus	1	0.0032	6.18* (≈2.0%)
Nitrogen x potassium	1	0.0018	3.48 n.s.
Nitrogen x oil	4	0.0080	15.44***
Phosphorus x potassium	1	0.0006	1.16 n.s.
Phosphorus x oil	4	0.0014	2.70*
Potassium x oil	4	0.0009	1.74 n.s.
Nitrogen x phosphorus x potassium	1	0.0003	0.58 n.s.
Nitrogen x phosphorus x oil	4	0.0002	0.39 n.s.
Phosphorus x potassium x oil	4	0.0006	1.16 n.s.
Nitrogen x phosphorus x Potassium x oil	4	0.0003	0.58 n.s.
E.M.S. = 0.000754	d.f. = 39		

Table 36a: Effect of nitrogen, phosphorus and oil additions to soil on the shoot dry matter yield of Tama ryegrass (crop 1). Subscript o denotes no added nutrient and subscript 1, added nutrient.

(i) Main effect-nitrogen*** † Means of 120 samples

Shoot dry matter yield/plant(g)†

N_o = 0.15

N_1 = 0.21

S.E. = ± 0.004342 L.S.D. (5%) = 0.01

(ii) Main effect-phosphorus *** †Means of 120 samples

Shoot dry matter yield/plant(g)†

P_o = 0.20

P_1 = 0.17

S.E. = ± 0.004342 L.S.D. (5%) = 0.01

(iii) Main effect-oil*** † Means of 48 samples

	Initial oil application rate (t/ha)				
	0	56	112	168	224
Shoot dry matter yield/ plant (g) †	0.20	0.20	0.18	0.16	0.18

S.E. = ± 0.006865 L.S.D. (5%) = 0.02

(iv) Interaction-nitrogen x oil*** † Means of 24 samples

	† Shoot dry matter yield/plant(g)				
	Field oil application rate (t/ha)				
	0	56	112	168	224
N_o	0.20	0.19	0.14	0.10	0.14
N_1	0.21	0.21	0.23	0.21	0.22

S.E. = ± 0.009708 L.S.D. (5%) = 0.03

(v) Interaction-nitrogen x phosphorus * † Means of 60 samples

	Shoot dry matter yield/plant(g) †	
	N_o	N_1
P_o	0.16	0.24
P_1	0.14	0.19

S.E. = ± 0.006140 L.S.D. (5%) = 0.02

(vi) Interaction - phosphorus x oil*. Means of 24 samples.

	Shoot dry matter yield/plant(g)				
	Field oil application rate (t/ha)				
	0	56	112	168	224
P_o	0.21	0.21	0.21	0.18	0.19
P_1	0.19	0.19	0.16	0.13	0.18

S.E. = 0.009708 L.S.D. (5%) = 0.03

Table 37: Effect of micronutrient addition to oiled Timaru silt loam on the shoot dry matter yield of Tama ryegrass (crop 1).

Analysis of variance table.

Source	d.f.	Mean Square	F
Micronutrients	1	0.0020	1.22 n.s.
Oil	4	0.0049	2.99 n.s.
Micronutrients x oil	4	0.0002	0.12 n.s.
E.M.S. = 0.0016		d.f. = 10	

deficiency of plant available nitrogen rather than some affect ascribable to oil and oil alteration products. Inorganic nitrogen added to such soils alleviated symptoms of nitrogen deficiency. It was considered on available evidence that oil addition had promoted immobilization of nitrogen.

Shoot dry matter yields from soils amended with phosphorus were significantly reduced on 112 and 168 t/ha soils while those from 0, 56 and 224 t/ha soils were unaffected. Where both N and P amendments were made, the apparent inhibitory effect of phosphorus as indicated by shoot dry matter yield was removed but mean yield from the five soils was approximately 21 percent less than pots to which only nitrogen had been added. Nitrogen (225 kg/ha) and phosphorus as superphosphate and as a component of waste lubricating oil equivalent to 145 kg/ha P were applied to the 168 t/ha oiled field plots to give an N:P ratio of 1.0 : 0.6 which was considerably lower than that of microbial tissue 1.0 : 0.3 (Sistrom 1962), suggesting that nitrogen in the oiled soil would have been preferentially immobilized by microorganisms assuming available carbon compounds had been present.

Consideration of the phosphorus levels likely to be present in those soils associated with waste lubricating oil suggested that phosphorus in the P amended pots was at a concentration near toxic to plant growth. Rossiter (1952) obtained reduced yield for oats grown on a coarse sand (pH 5.3-5.5) deficient in lime and nitrogen

and to which phosphorus (1132 kg/ha as super phosphate) had been added. Percent total phosphorus of the oat tops after 101 d growth was 4.51. Application of sodium nitrate (503 kg/ha) to the soil increased yield more than 20 fold and decreased phosphorus concentration more than 3 fold. No inhibitory effect of NO_3-N on phosphorus absorption was observed and the promotion of plant growth by added nitrogen was considered to produce a dilution effect whereby the internal concentration of phosphorus did not become critically high. Only one crop was grown and the same effect was produced by a large range of soluble phosphorus salts. A similar pattern of yield was obtained for the present experiment.

It is difficult to explain the result obtained for phosphorus amended 224 t/ha oiled soil but it is conceivable the oil residues may have suppressed uptake of phosphorus on such soils.

A non-significant F test for the second analysis (Table 37) indicated that the trace element treatment had not affected shoot dry matter yield.

The long term effects of nutrient amended soils to which waste lubricating oil had been added or not was investigated by growing a second crop of Tama ryegrass. No further tiller growth could be obtained from the first plants because they had reached the flowering stage at the time of harvesting. Experimental details were the same as for crop 1.

Table 38: Effect of nitrogen, phosphorus , potassium and oil on the shoot dry matter yield of Tama ryegrass grown on Timaru silt loam (crop 2).

Analysis of variance table

Source	d.f.	Mean square	F
Nitrogen	1	0.1604	121.42***
Phosphorus	1	0.0026	1.97 n.s.
Potassium	1	<0.00005	<< 1.00 n.s.
Oil	4	0.0148	11.20***
Nitrogen x phosphorus	1	0.0007	0.53 n.s.
Nitrogen x potassium	1	<0.00005	<< 1.00 n.s.
Nitrogen x oil	4	0.0012	0.91 n.s.
Phosphorus x potassium	1	0.0013	0.98 n.s.
Phosphorus x oil	4	0.0018	1.36 n.s.
Nitrogen x phosphorus x potassium	1	0.0015	1.14 n.s.
Nitrogen x phosphorus x oil	4	0.0012	0.91 n.s.
Nitrogen x potassium x oil	4	0.008	0.61 n.s.
Phosphorus x potassium x oil	4	0.009	0.68 n.s.
Nitrogen x phosphorus x Potassium x oil	4	0.0011	0.83 n.s.
E.M.S. = 0.001287	d.f. = 39		

Table 38a: Effect of nitrogen and oil additions to soil on the shoot dry matter yield of Tama ryegrass (crop 1).

Subscript o denotes no added nutrient and subscript 1 added nutrient.

(i) Main effect nitrogen*** † Means of 120 samples

Shoot dry matter yield/plant †

N_o = 0.19

N_1 = 0.28

S.E. = ± 0.005672 L.S.D.(5%) = 0.02

(ii) Main effect oil*** † Means of 48 samples

	Initial oil application rate (t/ha)				
	0	56	112	168	224
Shoot dry matter yield/plant †	0.22	0.21	0.21	0.26	0.27

S.E. = ± 0.008969 L.S.D. (5%) = 0.03

Table 39: Effect of Micronutrient addition to oiled Timaru silt loam on the shoot dry matter yield of Tama ryegrass (crop 2).

Analysis of variance table

Source	d.f.	Mean square	F
Micronutrients	1	< 0.00005	0.02 n.s.
Oil	4	0.0012	0.50 n.s.
Micronutrients x oil	4	0.0002	0.08 n.s.
E.M.S. = 0.0024		d.f. = 10	

(c) Results (Crop 2)

Shoots were harvested from all plots on day 33, oven dried (105°C) for 24 h and weighed. Analysis of variance of results obtained are shown in Table 38. The main points of the first analysis are summarized as follows: yields for main effect oil were not significantly different for 0, 56, 112 t/ha soils or 168 and 224 t/ha soils (not amended with nutrients). In contrast to crop 1 results, the addition of nitrogen increased shoot dry matter yield by the same amount on all soils. Interaction between phosphorus and oil for crop 2 was not significant. Shoot dry matter yield for the crops grown on unamended soils differed in the following ways: crop 1 yield from 168 and 224 t/ha soils was less than that obtained from 0 and 56 & 112 t/ha soils while for crop 2 that from 168 and 224 t/ha soils exceeded that from 0, 56 and 112 t/ha soils. Total yield for the two crops on unamended soils is given in Table 40.

Table 40: Total shoot dry matter yield of Tama ryegrass (crops 1 & 2) grown on unamended oiled Timaru silt loam.

	Field oil application rate (t/ha)				
	0	56	112	168	224
Total shoot dry matter yield crops 1 & 2 (g)	2.19	2.26	1.87	1.91	2.13

The only outstanding results appeared to be for 112 and 168 t/ha soils the shoot dry matter yield for which was about 12 percent less than that obtained for the other three. Main effect phosphorus and its interaction with nitrogen and oil were not present in the second crop. The pots were not free draining, root residues left after the first crop would present a high carbon content and therefore, phosphorus and nitrogen would have been immboilized in addition to that removed by the shoots. A change in the amount of plant available nitrogen in the soils since the first crop was harvested was suggested by the absence from crop 2 of a significant N x oil interaction. Similar total shoot dry matter yields obtained for the two crops on the 5 soils not amended with nutrients suggested that the oil residues present had little detrimental effect on shoot growth of *Lolium multiflorum* under growth cabinet conditions. As for the first crop, trace elements had no effect on shoot dry matter yield (Table 39).

(2) Percentage Nitrogen in Shoots of *Lolium multiflorum* var. Westerwolds "Grasslands Tama" grown on oiled Timaru silt loam. Exchangeable NO_3, NO_2 and NH_3 and 'Plant Available' Phosphorus of and Rate of Oil Loss from, Oiled Timaru Silt Loam.

To provide further information on the possible effects of oil residues on shoot dry matter yield, determinations were made of the percentage nitrogen in the Tama ryegrass shoots, exchangeable NO_3^-, NO_2^- and NH_4^+ of

soils from the pot experiment and 'plant available' phosphorus content and the rate of oil loss from those soils.

(a) Method

For percent nitrogen determination of the herbage, total shoot dry matter of crops 1 and 2 from each soil (not amended with nutrients) was ground in a Watson blendor to pass through a 1.0 mm screen. Triplicate samples of about 2.5 mg were weighed to $\pm$ 0.001 g and analysed by a Coleman Dumas analyser. Exchangeable NO_3, NO_2 and NH_3 were determined for incubated unoiled and oiled soils using a method of Bremner (1965a) involving steam distillation as given earlier. Soxhlet extractable oil content of the soils was determined using the method given on page 62 . Extractable oil was measured for both unamended and N.P. amended potted soils to determine whether the nutrient amendements had affected the loss of oil over the period of the bioassay experiment. The soils were of calcareous nature due to the addition of lime (p. 67) and therefore 'plant available' phosphorus was extracted using Morgan's glacial acetic acid sodium acetate reagent (Morgan 1937). Phosphorus determinations were made by a colorimetric method (Allen et al. 1974).

(b) Results

Results are presented in Tables 41 and 42. Figure 19 shows the linear inverse relationship between percent nitrogen

Table 41: (a) Percentage nitrogen in Tama ryegrass shoot dry matter grown on oiled Timaru silt loam.

Soil plot	Field Oil Application Rate (t/ha)	Total Shoot d.m. yield crops 1 & 2 (g)	Percent N in shoot d.m. crops 1 & 2 *1	Total N removed by shoots crops 1 & 2 (g)
224	0	3.62	1.72	6.43×10^{-3}
118	56	3.53	1.92	6.76×10^{-3}
123	112	3.21	2.30	7.39×10^{-3}
122	168	3.41	2.13	7.25×10^{-3}
219	224	3.65	1.84	6.70×10^{-3}

(b) Exchangeable nitrogen content of oiled Timaru silt loam (after bioassay).

$\bar{X}$ Exchangeable NO_3, NO_2 and NH_3 $\mu g/g$ *†2	$\bar{X}$ Exchangeable NO_3, NO_2 and NH_3 kg/ha
98.0	184.0
37.8	71.0
32.2	60.3
28.0	52.5
42.0	78.8

* means of 3 replicates

† assumes 1 ha soil to a depth of 9-12 cm weights 1.88×10^6 kg

1 C.V. = 20.4

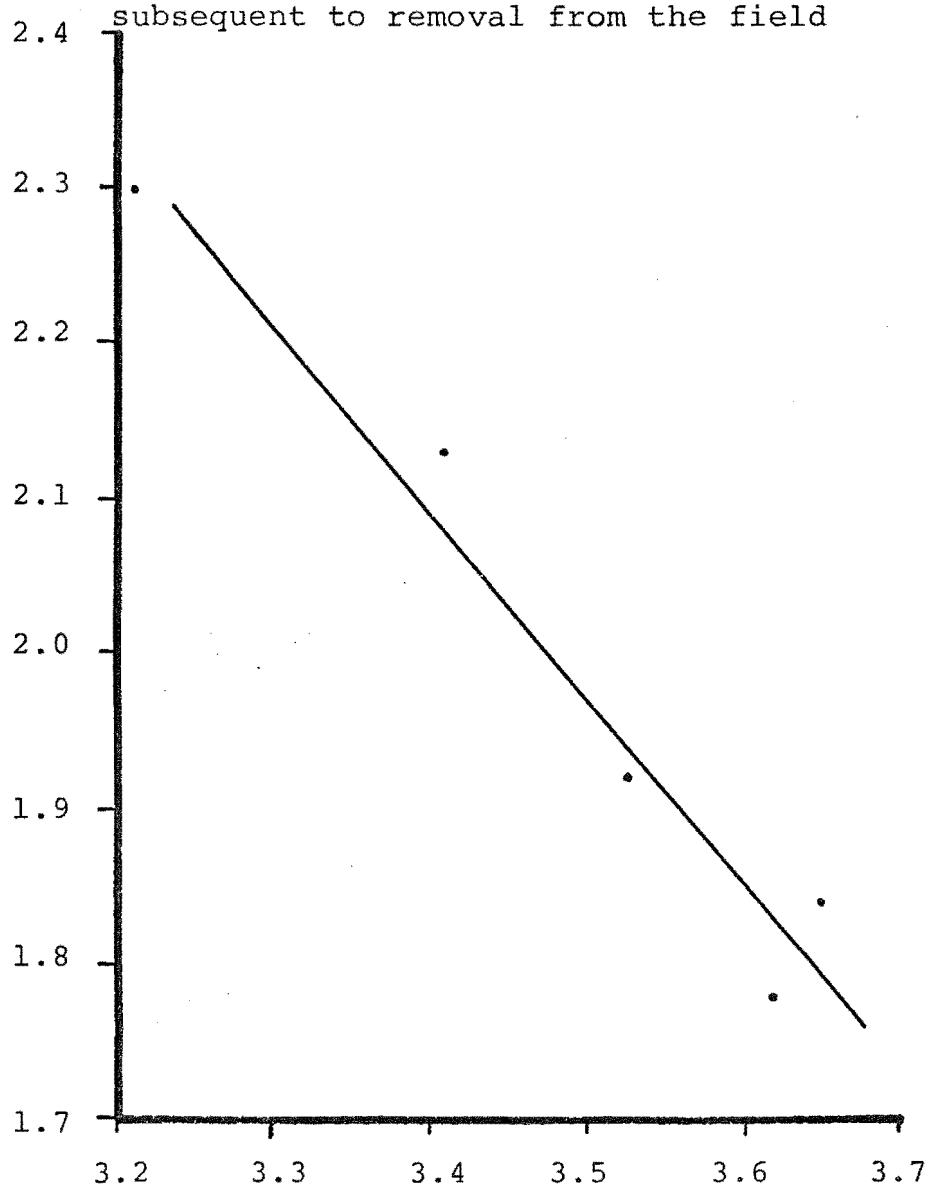
2 C.V. = 17.0

Table 42: 'Plant available' phosphorus in and rate of loss of waste lubricating oil from oiled Timaru silt loam (after bioassy).

Soils not amended with nutrients						Soils amended with nitrogen and phosphorus
Soil plot	Field waste lubricating oil application rate (t/ha)	Mean 'plant available' P mg/100 g air dried soil	mean 'plant available' P kg/ha †	total P applied to soil kg/ha	mean rate of loss of soxhlet extractable oil g/kg/month	mean rate of loss of soxhlet extractable oil g/kg/month
224	0	1.68	31.4	68	N.D.	N.D.
118	56	1.42	26.5	94	1.3×10^{-2}	0.9×10^{-2}
123	112	2.03	38.2	120	0.6×10^{-2}	0.8×10^{-2}
122	168	2.40	45.2	145	0.4×10^{-2}	N.D.
219	224	2.13	40.0	171	0.9×10^{-2}	1.3×10^{-2}

† assumes 1 ha soil to a depth of 9-12 cm weighs 1.88×10^{6}kg.

Figure 19: Relationship between percent nitrogen of shoot dry matter and shoot dry matter yield of Tama ryegrass grown on oiled Timaru Silt Loam. Crops 1 & 2. combined. No nutrient amendments made to the soils subsequent to removal from the field



Equation of line $y = -1.189 x + 6.134$.

$F = 71.9^{***}$

S.E. of regression coefficient

= 0.01402

of the shoot dry matter and total shoot dry matter yield of the two crops. Approximately equal amounts of nitrogen were removed from all soils by the two crops (Table 41). These results suggested that nitrogen was not limiting growth on the oiled soils. Concentration of lead applied as a component of waste lubricating oil to plots of various oil application rates was calculated on the basis of a lead concentration for the oil of 1.58 percent (Appendix 1). Analysis of the inorganic soil nitrogen (p200) did not distinguish between NH_3-N and NO_3-N. One possible explanation for the observed stimulation on both soils by an additional nitrogen source was that concentration of lead in the soil was sufficient to have reduced or inhibited biological oxidation of NH_3-N to NO_3-N. Under such conditions, the nitrogen requirements of Tama ryegrass would have been satisfied by NO_3-N in the nutrient solution. Further research would be needed to confirm or otherwise this hypothesis.

Immobilization of nitrogen in the oiled soils was suggested by the relatively high inorganic nitrogen concentration of the unoiled control (98 µg/g or 184 kg/ha). Air-drying also causes loss of nitrogen (L. Greenfield pers. comm. 1976); consequently it was unusual to find a large amount of inorganic nitrogen in air-dried soils. 'Plant available' phosphorus on the oiled soils increased with increased applied phosphorus up to a field oil application rate of 168 t/ha. The value obtained for the 224 t/ha soil was lower than expected and was about equal to that of

112 t/ha. Phosphorus immobilization in the oiled soils was suggested by the phosphorus concentration obtained for the 56 t/ha plot which was approximately 20 percent less than that of the unoiled control.

As mentioned elsewhere, the presence of waste lubricating oil residues in soil might have been expected to affect soil moisture retention and as a result, plant growth. Previous experiments indicated that oil residues in soil had little detrimental effect on shoot dry matter yield of Tama ryegrass but because they may have affected soil moisture retention capacity the possibility was investigated.

(3) Effect of Waste Lubricating Oil on Moisture Retention of Timaru Silt Loam

(a) Method

Approximately 1 kg of each of the field soils used in the previous experiment was weighed into a 1 ℓ plastic free draining pot and brought to field capacity with tap water and the moisture retention capacity of the 5 replicate samples of each soil was determined for a range of pressures using a pressure plate moisture extractor (Soilmoisture Equipment Corporation P.O. Box 30025, Santa Barbara, California 93105, U.S.A.).

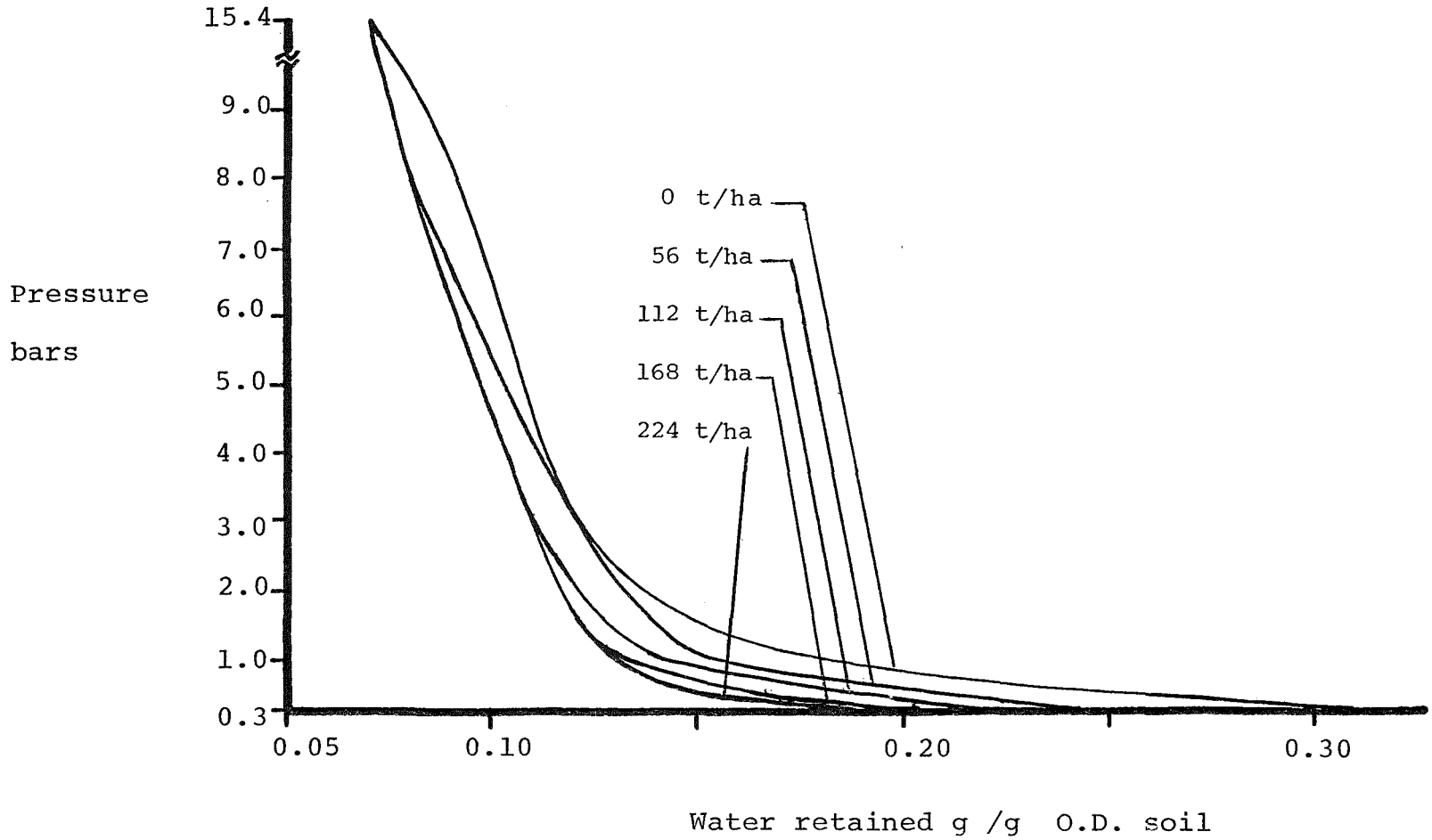
(b) Results

Results given in Table 43 and Figure 20 indicated that at low pressures the presence of oil residues lowered the moisture retention capacity for all application rates. The only outstanding results appeared to be for 0.3 and 1.0 bar, pressures at which soil moisture retention capacity decreased with increased oil application rate. The soil at these pressures was near field capacity and it was therefore unlikely that soil moisture retention capacity would have had a deleterious effect on plant growth under field conditions.

Table 43: Soil moisture retention of Timaru silt loam 90 weeks after the application of waste lubricating oil.

Plot no.	Initial oil application rate (t/ha)	Pressure (bars)				
		0.3	1.0	3.0	8.3	15.0
224	0	0.31	0.18	0.12	0.09	0.07
118	56	0.25	0.15	0.12	0.08	0.07
123	112	0.22	0.14	0.11	0.08	0.07
122	168	0.21	0.13	0.11	0.08	0.07
219	224	0.19	0.13	0.11	0.08	0.07

Figure 20: Soil moisture retention of Timaru silt loam 90 weeks after the application of waste lubricating oil.



CHAPTER V

THE UPTAKE OF LEAD FROM OILED TIMARU SILT LOAM BY *Lolium multiflorum* LAM. VAR. WESTERWOLDS "GRASSLANDS TAMA"

Lead residues present in soil used for the disposal of waste lubricating oil may conceivably be taken up by plants grown on such sites thereby presenting a possible health hazard to man and grazing animals. Lead which occurs in waste lubricating oil is derived almost totally from the organo-lead compounds added to petrol as anti-knock agents (Shell Oil New Zealand Ltd. pers. comm. 1973). The metal is usually added as the tetra-ethyl, tetra-methyl or di-ethyl-di-methyl form although the residues occurring in the engine oil are almost all inorganic and may include $PbBr_2$, $PbCl_2$, $PbO.PbCl_2$, $2PbO.PbBr_2$, PbO, $PbO.PbSO_4$ and $PbSO_4$ (Shell Oil New Zealand Ltd. pers. comm. 1973). The variety and relative amounts of the compounds present vary according to engine operating conditions (Shell Oil New Zealand Ltd. pers. comm. 1973). Time did not permit an investigation of all those plant genera consumed by man and grazing animals and which might remove lead from soil. The approach adopted for this study was to select a common pasture species known to take up lead into its shoot system, grow it on the oily residues of soils from the Timaru land disposal site and to determine the lead concentration of the shoots.

Several plant species have been shown to take up lead from soil or solution and to deposit some of it in the shoot system (*Alfalfa sp.*, MacLean et al., 1969; *Bromis enermis* Leyss, Marten and Hammond 1966; *Luctuca sativa L.* and *Avena sativa L.*, John et al. 1972; *Hordeum sp.*, Keaton 1937; *Glycine max.*, *Triticum sp.*, and *Avena sp.*, Cox and Rains 1972; *Lolium perenne* L. var. S23, Jones et al. 1973).

Perennial ryegrass (*Lolium perenne*) which is a common component of New Zealand pastures, readily available and suitable for bioassy experiments was selected for a lead uptake study. The species was used by Jones (1972) to study lead uptake from solution culture. He found appreciable amounts of the metal in both its shoot and root system (14.9 and 168.4 p.p.m. respectively on a dry weight basis) for 35 d old plants when supplied with approximately 250 µg Pb/plant for 3 d.

An experiment was performed to determine the suitability of *Lolium perenne L.* "Grasslands Ruanui" as a bioassay plant and to examine the suitability of various dry ashing methods for lead determination in plant tissue.

Ryegrass seeds surface sterilized in 3 percent hypochlorite were germinated in petri-dishes and four days later 10 of the seedlings were planted in each of six 1 ℓ plastic pots filled with a coarse/fine sand mix. The sand was first washed several times with tap water. Three pots were placed in each of 2 large petri dishes to which 500 ml of nutrient solution (see Appendix 3) was

added. Black polythene sheet was fitted over the solution surface to reduce evaporation. A further 50 ml of solution was poured over the sand in each pot before dishes and pots were placed in a controlled growth cabinet (room temperature 25°C, 16 h d, light source fluorescent tubes and tungsten bulbs). Mean relative humidity was about 70 percent but fluctuated during the growth period. Nutrient solution was changed approximately weekly but otherwise maintained.

Ninety-four days after the seedlings had been transferred to the sand medium a further litre of nutrient solution containing 5000 µg of lead as $Pb(NO_3)_2$ complexed with di-sodium E.D.T.A. was added to each dish. The removal of any dead plants (presumably killed through over-crowding) immediately prior to the addition of lead resulted in a mean application rate of 316 µg Pb/plant to the remaining plants.

On day 100 when the lead containing nutrient solution had been used up, a further 2 ℓ of 448 ppm N nutrient solution without lead was added to the dishes and on day 105 the plants were carefully removed from the pots, washed and separated into root and shoot. The shoots were oven-dried (24 h 105°C) and ground in a Watson blendor to pass through a 1.0 mm screen.

I. ANALYSIS OF *Lolium perenne* L. "GRASSLANDS RUANUI" SHOOTS FOR LEAD BY ATOMIC ABSORPTION SPECTROSCOPY.

(1) Preparation

Several methods have been used to prepare oven-dried ground plant tissue for lead analysis. These include one or more dry ignitions at a range of temperatures with or without an ashing aid and wet ignition using a variety of combinations of inorganic acids. Such methods have been reviewed by Gorsuch (1959). Recovery rates for wet oxidation techniques vary with the acid combination used and the chemical composition of the material under test while for dry oxidation, ashing temperature, number of ignitions, ashing aid used, chemical composition and history of the crucible and chemical composition of the test material are important factors.

There are few reports of quantitative lead recovery from vegetation. Webber (1972) compared the relative lead recovery rates for a variety of plant tissues using a range of wet and dry-ashing preparative methods. Webber claimed to have obtained complete recovery of lead from a range of plant tissues which were ashed at 430^{o}C but no spiking experiments were carried out nor any indication of an independent analysis given.

Parker et al. (1974) when determining the lead content of orchard leaves found that a single ashing at 400^{o}C for 4 h gave 96-98 percent recovery for lead added in the form of $Pb(NO_3)_2$ over a range of 0-10 µg. A similar

percentage recovery was obtained for the orchard leaves, the expected concentration of which was based on National Bureau of Standards certification.

Recovery rate for lead in plant tissue varies from one plant tissue to another as demonstrated by Webber (1972) and it could not be assumed that ryegrass and orchard leaves would behave similarly. Where recovery rates have been determined from spiking experiments the compound of lead added as a spike is of importance. To be of use, the behaviour of the spike lead when ashed must reflect that of the plant tissue. Ashing temperature and time required for complete ashing also vary with the plant species under investigation (Gorsuch 1959).

Determination of lead recovery rate for ryegrass shoots was based on the observation by Gorsuch (1959) that the percentage of spiked lead recovered from plant tissue varied in the presence of different ashing aids. Samples of plant tissue are ashed with a chosen lead spike compound at various concentrations and separately with a range of ashing aids. Providing, for different ashing aids, the percentage change in recovery rate of the spike compound reflects that in the concentration of plant lead, the lead spike compound may be considered to behave in a manner similar to lead from the plant tissue.

(2) Procedure

Samples (0.2g ± 0.005) of oven-dried ground ryegrass shoots grown in sand culture (see p209) were weighed into previously unused 30 ml porcelain crucibles and each spiked with 1 ml of lead as lead nitrate in approximately 2 M nitric acid†. Lead nitrate was chosen because it has been shown by Gorsuch (1959) not to volatolize at a temperature of 650°C. Lead was added at 6 concentrations (0.0, 1.0, 10.0, 50.0, 100.0 and 500.0 µg/ml) to provide five replicates. The lead concentrations were chosen to cover the likely lead concentrations of the bioassay plants (later to be grown on the oiled Timaru soil) and to determine the effect of lead concentration on recovery rate. A further 5 crucibles containing only nitric acid were included as blanks. Five hundred µg Pb/ml stock solution provided a set of standards which were stored in polypropylene containers.

Lids were placed on the crucibles and the shoot samples ashed in a muffle furnace at 460°C for 16 h. Two ml of 2 M nitric acid was added to each crucible of cooled ash, warmed to boiling point and diluted in volumetric flask to 10 ml with deionized water. Solutions were transferred to 15 ml P.V.C. capped tubes and stored in a refrigerator. Blank samples were similarly treated.

† 'Aristar' nitric acid in deionized water was used for all sample preparation. All glassware was rinsed in laboratory grade 2 M HNO_3 deionized water.

II. LEAD DETERMINATION BY ATOMIC ABSORPTION SPECTROSCOPY

Atomic absorption spectroscopy has been used by workers for the determination of lead in plant material (Fletcher 1972, Parker et al. 1974). Billings (1965) found that solutions of plant material (which contain large amounts of dissolved solids) absorb strongly in an air-acetylene flame at the 217 nm wavelength, the most sensitive lead line. Because matrix absorption and that due to an impurity of the test element in the matrix are indistinguishable without scanning, a hydrogen continuum lamp has been used to measure background absorbance by scanning wavelengths adjacent to the line emitted by the Ne-Pb hollow cathode lamp (Fletcher 1971). These corrections are valid if the background absorbance at the precise wavelength of the elemental line is the same as the average absorbance in the wavelength interval passed by the monochromator.

(1) Procedure

Lead determinations were made using a Varian Techtron AA-5 Atomic Absorption Spectrometer and results recorded on a Yokogama chart recorder (chart speed 2 cm/min). Operating conditions were: Ne-Pb lamp current 6.8 mA; hydrogen continuum lamp current 14 mA; fuel (acetylene) flow 1.9, (arbitrary unit on the techtron gas control unit type GCU-5; support (air) pressure 104.6 kPa ; analytical line λ = 217.0 nm; spectral band pass 0.2 nm; exhaust control $\frac{2}{3}$ closed.

Three replicate readingswere made for each sample using the Ne-Pb lamp and background absorbance measured by the hydrogen continuum lamp was subtracted directly. Results are recorded in Table 44.

Similar recovery rates were obtained for the 4 highest additions of lead while that for the 1.0 µg amendment was considerably lower. A possible explanation for the lower lead recovery rates is that advanced by Gorsuch (1959) who concluded that the retention of elements on the surface of the ashing crucible of vitreous silica or porcelain composition was responsible for losses during dry ashing;"... the obvious reaction is between the oxide of the element of interest and the ashing vessel to produce a complex silicate so causing a loss." He considered lead to be one of the most reactive oxides.

Results shown in Table 45 indicate that more lead was recovered from spiked and unspiked samples where 10 mg magnesium nitrate, ground to pass a 210µ sieve was used as an ashing aid. Unless otherwise stated, experimental details for this and subsequent experiments remained the same. The percentage increase in the lead content obtained for the shoots was similar to that obtained for the spike at all concentrations suggesting lead nitrate to be a suitable spike. Loss of the 1.0 µg amended samples from the magnesium nitrate experiment precluded full comparison with those samples prepared without an ashing aid.

Table 44 : Lead recovered from ground shoots of Ruanui ryegrass grown in sand culture with a nutrient solution containing lead. Shoots spiked with lead nitrate. † Means of 5 0.2g ± 0.005 samples.

	Controls		Spike lead added (µg)				
	Blank	Plant tissue only	1.0	10.0	50.0	100.0	500.0
Mean lead† content (µg)	N.D.	2.446	3.00	9.233	35.125	67.733	333.566
Mean weight of spike lead recovered (µg)	-	-	0.553	6.787	32.678	62.287	304.100
Mean weight of spike lead recovered as a percentage of spike lead added	-	-	55.3	67.9	65.4	65.3	61.8
C.V.	-	15.8	4.6	6.3	6.2	7.6	7.5

Table 45: Lead recovered from ground shoots of Ruanui ryegrass grown in sand culture with a nutrient solution containing lead. Shoots spiked with lead nitrate - ashing aid, magnesium nitrate. † means of 5 0.2 g ± 0.005 samples.

	Controls		Spike lead added (µg)			
	Blank	Plant tissue only	10.0	50.0	100.0	500.0
Mean lead content (µg)†	N.D.	2.820	10.510	40.050	81.410	409.900
Mean weight of lead recovered (µg)	-	-	7.69	37.23	78.59	407.08
Mean weight of spike lead recovered as a percentage of spike lead added	-	-	76.9	78.5	78.6	81.4
C.V.	-	15.3	4.8	6.2	4.1	2.5
Percentage increase in spike lead recovered c.f. no ashing aid	-	11.6	12.0	12.0	12.0	13.2

An experiment to determine whether the pattern of lead recovery could be repeated was abandoned because of accidental loss of the crucibles and because insufficient plant sample remained to repeat all the recovery investigations, a third experiment was performed to determine the effect of a second ashing aid (boric acid) on the recovery rate of lead from lead spiked plant samples.

Lead as lead nitrate was used to spike further 0.2 g ± 0.005 samples of oven dried, ground Ruanui ryegrass shoots with 0,5 and 10 µg of lead. Additions were based on the amount of lead recovered from the shoots in a previous experiment. Ten mg of boric acid was added to each of the samples which were ashed at 650^{o}C for 16 h. At a lower temperature a glass-like bead (insoluble in nitric acid) was formed.

Results in Table 46 show almost complete recovery of the 10 µg lead spike but only about half of the 5 µg amendment, a pattern similar to that obtained for spiked samples prepared without an ashing aid. In contrast, the lead content of the unspiked shoot material increased by 66 percent when ashed with boric acid. Behaviour of the lead spike did not therefore reflect that of the shoots.

To obtain further information on possible losses of lead by crucible retention and the effectiveness of ashing aids, 10 further samples were prepared, five of which were spiked with 5 µg as lead nitrate.

Table 46: Lead recovered from ground shoots of Ruanui ryegrass grown in sand culture with nutrient solution containing lead. Shoots spiked with lead nitrate - ashing aid, boric acid.

† Means of 5 0.2 g ± 0.005 samples.

	Controls		Spike lead added (µg)	
	Blank	Plant tissue only	5.0	10.0
Mean lead content (µg) †	N.D.	4.05	6.58	13.60
Mean weight of spike lead recovered (µg)	-	-	2.53	9.55
Mean weight of spike lead recovered as a percentage of lead spike added	-	-	50.6	95.6
C.V.	-	32.9	24.1	17.4

Crucible sets used were the same as for the previous experiment and each sample received 10 mg of magnesium nitrate. Samples were ashed at 460°C for 16 h.

Results (Table 47) indicated complete recovery of the 5 µg lead spike in contrast to the 50 percent recovered for the previous experiment with boric acid as an ashing aid and the 55 percent recovery of the 1 µg lead spike of the first experiment (no ashing aid). Repeated loss of lead spike

Table 47: Lead recovered from ground shoots of Ruanui ryegrass grown in sand culture with nutrient solution containing lead. Shoots spiked with lead nitrate - ashing aid, magnesium nitrate. † Means of 5 0.2 g ± 0.005 samples.

	Controls		Spike lead added (µg)
	Blank	Plant tissue only	5.0
Mean lead content (µg) †	N.D.	2.88	7.94
Mean weight of spike lead recovered (µg)	-	-	5.06
Mean weight of spike lead recovered as a percentage of lead spike added	-	-	104.0
C.V.	-	20.7	5.1

from previously unused crucibles and subsequent near complete recovery of 5 µg spike in the presence of $Mg(NO_3)_2$ and H_3BO_3 ashings suggested the explanation of Gorsuch (1959) (p.189) to be correct. The lead lost from the 5 µg lead spike for the boric acid experiment was presumably sufficient to have saturated available complexing sites on the crucible walls and to have permitted complete recovery of the 5 µg lead spike in the magnesium nitrate experiment. To test this hypothesis, two further 0.2 g ± 0.005 samples of remaining Ruanui ryegrass shoot sample were weighed into the '5 µg' crucibles and spiked with 5 µg of lead as lead nitrate). Ten mg of boric acid was added to each crucible which had previously been heated at 650°C for 16 h in a muffle furnace to remove any lead which might be lost above 460°C, the ashing temperature of the previous experiment.

Complete recovery of the 5 µg lead spike (Table 48) further supported the possibility that retention was responsible for the loss of lead spike at low concentrations and the contention of Gorsuch (1959) that once saturation of complexing sites on the crucibles has been achieved, complete recovery of the spike is possible. 'Non-saturated' crucibles used for dry ashing plant sample may result in an underestimation of plant lead concentration especially at low concentrations and it is therefore important that crucible history be included where the object of an experiment is to provide quantitative results. Collectively the experiments indicated that recovery of the lead spike in the presence of Ruanui ryegrass shoots was independent of the ashing aid used.

Table 48 : Lead recovered from ground shoots of Ruanui ryegrass grown in sand culture with nutrient solution containing lead. Shoots spiked with lead nitrate - ashing aid, boric acid. * Means of 6 0.2 g ± 0.005 samples. † means of 7 0.2 ± 0.005 samples.

	Controls		Spike lead added (µg)
	Blank	Plant tissue only *	5.0†
Mean lead content (µg)	N.D.	4.05	9.14
Mean weight lead recovered (µg)	-	-	5.09
Mean weight of spiked lead recovered as percentage of lead spike added	-	-	101.8
C.V.	-	32.9	7.2

The pattern of lead concentration obtained for the unspiked shoots was not always consistent with that for recovery of the lead spike as shown in Table 49 below.

Table 49: Effect of ashing aids on the recovery of lead from spiked and unspiked ground shoots of Ruanui ryegrass grown in sand culture with nutrient solution containing lead. Spike, lead nitrate. *Means of 6 0.2g ± 0.005 samples. † Mean of 5 0.2 g ± 0.005 samples.

	Ashing aid, boric acid	Ashing aid, magnesium nitrate
Mean lead content (µg) unspiked shoots	4.05	2.88
Percentage spike lead recovered from shoots spiked with 5 µg lead	101.8	104.0

The concentration of lead in the unspiked shoots in the presence of magnesium nitrate was lower than expected had the behaviour of the plant lead reflected that of the spike. Incomplete ashing of the shoot sample in the presence of magnesium nitrate may have reduced the lead yield from these samples. The amount of partially ashed shoot material adhering to the crucible walls appeared disproportionately low compared to the decrease in shoot lead concentration but may have fixed some of the lead thereby preventing its later dissolution in nitric acid.

III. UPTAKE OF LEAD BY SHOOTS OF *Lolium multiflorum* LAM. VAR. WESTERWOLDS "GRASSLANDS TAMA" GROWN ON OILED TIMARU SILT LOAM.

As mentioned elsewhere (p.160), *Lolium perenne* L. "Grasslands Ruanui" was considered too slow growing for the bioassay experiments and *Lolium multiflorum* Lam. var. Westerwolds "Grasslands Tama" was therefore used instead. Time prevented further lead uptake experiments with *L. multiflorum* and it was therefore assumed that because the two species belong to the same genus they would behave similarly.

(1) Methods

Lead concentration of Tama ryegrass shoots harvested from the bioassay experiment earlier described (p.183) was determined using boric acid as an ashing aid. Boric acid was selected because it permitted the highest recovery of lead from unspiked samples. The oven dried shoots from crops 1 and 2 were combined for each of soils 224^{o}† (o t/ha), 118^{o} (56 t/ha), 123^{o} (112 t/ha), 122^{o} (168 t/ha) and 219^{o} (224 t/ha) and ground in a Watson blendor to pass a 1 mm screen. Eight 0.2 g ± 0.005 plant samples from each soil were weighed separately into crucibles used for the previous ashing experiments. Ashing and lead determination procedures were as described above.

† Superscript 'o' denotes that the soils were not amended with nutrients subsequent to removal from the field.

(2) Results

Table 50 shows results for lead accumulation in shoots of Tama ryegrass plants and lead added to the soil as a component of waste lubricating oil. Oil from the tank used to supply the Timaru field experiment had a lead concentration of 1.58 percent (Shell Oil New Zealand Ltd. 1973a, Appendix 1).

Lead accumulation in shoots of plants grown on 56, 112 and 168 t/ha oiled soils was increased compared to that of the control soil. The increase of up to 35 percent was significant for only 56 and 168 t/ha soils and the analysis of further shoot samples would be necessary to obtain a significant difference for plants grown on 112 t/ha. Lead concentration of ryegrass shoots grown on the 224 t/ha oiled soil was approximately the same as that of the control. Phosphorus if present in the solution at a sufficiently high concentration may suppress absorbance at 217 nm (Varian Techtron 1971) and because 'plant available' phosphorus of the oiled soils increased with increased oil application (see p. 176) the possibility was investigated. Five ml of each of 3 replicate solutions representing shoots of plants grown on each of 5 soils was made up to 10 ml with 0.2M E.D.T.A. and analysed for lead. Lead concentration of those samples analysed was not affected and it was therefore possible that oil residues may have suppressed lead uptake by plants grown on the 224 t/ha oiled soil.

Table 50: Uptake of lead by shoots of Tama ryegrass grown on unoiled and oiled Timaru silt loam 89 weeks after oil application. † Means of 8 replicates. * Assumes 1 ha soil to a depth of 12 cm weighs 1.88×10^6 kg.

Rate of oil application to soil (t/ha)	Lead added to field plots as component of waste lubricating oil (p.p.m.)*	Lead content of shoots (µg/g)†
0	0	17.3
56	470	23.2
112	940	20.2
168	1410	23.4
224	1880	18.0

F = 3.23* S.E. = 1.85 L.S.D. (5%) = 5.4

CHAPTER VI

THE MICROBIAL DECOMPOSITION OF LUBRICATING OIL IN TIMARU SILT LOAM UNDER LABORATORY CONDITIONS

I. REVIEW OF PREVIOUS EXPERIMENTAL RESULTS

Results obtained for the Timaru field experiment indicate a decrease with time in the weight of 'hexane' soluble material present in all oil amended plots.

Evidence from subsequent laboratory experiments suggests that micro-organisms may have been at least partly responsible for the observed disappearance of oil. The evidence can be summarized as follows: increased microbial activity of the oil amended soil compared to that of the unoiled control soil; the ability of bacteria isolated from oil amended soil to extensively modify physically, pure paraffinic oil; evidence for the immobilization of phosphorus in oil amended soil and the lower nitrate, nitrite and ammonium ion content of oil amended soil. The above evidence for the microbial decomposition of oil is however, indirect and other laboratory experiments were considered necessary to further test the validity of this hypothesis.

II. MEASUREMENT OF THE MICROBIAL DECOMPOSITION OF LUBRICATING OIL IN SOIL UNDER LABORATORY CONDITIONS

(1) Review of Methods Available

Methods for the measurement of oil loss from and decomposition in soil have been reviewed elsewhere (pp 46-51) Most were considered unsuitable for a field experiment because of the large number of samples required and problems of data interpretation. For the purpose of laboratory experiments, several of these including soil respiration and gas chromatography can be used to provide evidence concerning the possible decomposition of mineral oil by micro-organisms.

The respiration of micro-organisms in soil was considered by Stotsky (1965) to be one of the earliest and most frequently used indices of soil microbial activity. The production of carbon dioxide was used as early as 1853 by Bossingault and Levey as a measure of in situ soil microbial activity. The method has since been used by workers to measure activity in both laboratory and field experiments Russell (1950), Waksman (1952), Stotsky and Norman (1961); Ross and Cairns (1978).

Respiration measurements have been found to correlate well with other parameters of microbial activity such as changes in organic matter content, nitrogen or phosphorus transformation, metabolic intermediates, pH, average microbial numbers and changes in soil weight, Stotsky (1960), Stotsky and Mortensen (1959), Stotsky and Norman (1961).

Bingeman et al. (1953) found that increased soil respiration rates following addition to the soil of C^{14}-

labelled *Alfalfa* and glucose resulted not only from decomposition of the added substrate but also in part from stimulated decomposition of the native soil organic matter.

Extrapolation of rates of mineralization obtained for a single labelled hydrocarbon to those for a complex mixture of hydrocarbons such as oil would be of doubtful validity because hydrocarbons are decomposed at different rates and in a mixture, often sequentially. Alternatively it might be possible to tritiate all hydrocarbons present in an oil and to measure the amount of tritiated water produced after a period of incubation. Whenever activity was measured it would be necessary to drive off and condense water from the soil. Because of the destructive nature of the technique, many replicates would be required for a time course experiment.

Experiments with mineral oils in liquid media have shown that respiration is not immediately stimulated following the first addition of the substrate. This lag phase was considered by van der Linden and Thijsse (1965) to result from the need for the soil microflora to adapt to provide the enzymes needed for decomposition of the added substrate. An immediate increase in the soil respiration rate following reapplication could thus be considered evidence that decomposition of the added substrate was at least partly responsible for any observed release of carbon dioxide.

The approach adopted for the present investigation

was to measure the respiration rate of soil amended with various oil treatments and to measure oil disappearance and investigate possible chemical modification of the oil by gas chromatographic analysis of soil extracts.

(2) Choice of a Technique for the Measurement of Soil Respiration

Both oxygen uptake and carbon dioxide evolution have been used to measure soil respiration (Stotsky 1965). Oxygen uptake techniques as a measure of carbon mineralization assume a respiratory quotient (ratio of the volume of carbon dioxide evolved to the volume of oxygen consumed of unity, (Smith and Brown 1932). For substrates which are poor in oxygen, the respiratory quotient will be less than unity. Hydrocarbons contain no oxygen and therefore oxygen uptake of oil amended soils would provide an indication of gross substrate alteration rather than the extent to which they have been mineralized. Carbon dioxide evolution because it provides an indication of the extent to which carbonaceous compounds have been mineralized was chosen as a measure of soil respiration for the present experiments.

Several techniques have been used to measure carbon dioxide produced by soil under laboratory conditions. These have been reviewed by Stotsky (1965), can be grouped on the basis of the method used to aerate the soil and include continuous air flow, intermittent air flow and no air flow. For continuous air flow, incubation vessels are continuously

aerated with a stream of carbon dioxide free air. Evolved carbon dioxide is flushed from the soil and passed through separate vessels containing alkali. The carbon dioxide absorbed by the alkali is then determined by conductometry, colorimetry, volumetry, gravimetry, turbidometry, or titrimetry. The concentration of evolved carbon dioxide may also be measured directly by infra-red absorption, gas chromatography or mass spectrometry.

The disadvantage of the continuous aeration technique is that where the desired moisture content is less than field capacity it is difficult to maintain a constant water content. Unless the air passing through the soil has the same humidity as the soil atmosphere which is in equilibrium with the soil, the air stream will remove water until the soil is air dry. Under these circumstances variations in respiration rate may merely reflect differences in soil moisture content.

For intermittent aeration, a carbon dioxide free air stream is used to periodically flush evolved carbon dioxide from the soil into an alkali trap. This method was considered by Stotsky (1960) to more closely approximate field conditions because some carbon dioxide is present in the soil atmosphere between flushings.

Possibly the most commonly used technique for measuring soil respiration is in the absence of an airflow. Samples of soil are incubated in a sealed chamber containing a vessel of alkali. Respired carbon dioxide is absorbed

by the alkali and the amount determined periodically by titration or other means. The technique has the advantages of being simple, less time consuming and requiring less space than the other two described. It does however have several limitations. Where microbial activity in a soil is high, oxygen may become limiting after a short period of time. This problem can be minimized by opening the chambers periodically thereby permitting the soil to re-equilibrate with the atmosphere and by selecting an incubation vessel with a large volume relative to that of the soil. Moisture is also lost to the atmosphere when the chambers are opened but can be replaced as needed. Provided the moisture fluctuations are small, the effect on the system should be minimal.

It has been suggested (Witkamp and Frank 1969) that reduced pressure inside respirometers caused by the absorption of carbon dioxide by alkali in a static system accelerates carbon dioxide diffusion from the soil and draws air containing high concentrations of carbon dioxide to the surface. Removal and absorption by alkali of high concentrations of carbon dioxide trapped in air pockets may also lead to elevated rates of carbon dioxide evolution. (Macfadyen 1971). This limitation is common to all three techniques.

Smith and Brown (1933) considered that the rate of carbon dioxide evolution into soil air may not accurately reflect the rate of production because the concentration of

evolved carbon dioxide is a function not only of the rate of production but also of the rate of escape. The solubility of carbon dioxide at one atmosphere pressure and temperatures of 15°C- 25°Cis low, approximately 1.66 mg CO_2/ml H_2O at 20°C, Perry and Chilton (1973). In a static system the soil solution would therefore become saturated with carbon dioxide in a relatively short time. Under these conditions rate of absorption should approximate rate of production.

There are conflicting reports concerning how closely soil respiration rates determined under laboratory conditions using the static chamber technique reflect those occurring under field conditions. Witkamp and Frank (1969) claimed that the static chamber method results in an over-estimate of field soil respiration rates whereas Coleman (1979) found an under-estimate. In another investigation, Muller et al. (1936) found that the technique provided a better estimate than that obtained using aeration techniques. De Jong et al. (1979) measured soil respiration rates under field conditions and found similar respiration rates for static chamber and soil carbon dioxide profile techniques. The soil carbon dioxide profile technique was based on in situ measurements of the soil carbon dioxide and an estimate of the appropriate diffusion coefficients.

Despite the limitations of the static chamber technique it does provide valuable information concerning relative soil respiration rates. For this reason and

because of the advantages described above, the technique was used for the experiments carried out in the present investigation.

(3) Preliminary Experiments

(a) To Determine the Effect of Alkali Surface Area on the Amount of Absorbed Carbon Dioxide. For a comparison of soil respiration rates to be valid it is essential that evolved carbon dioxide be absorbed by the alkali trap at a rate equal to its rate of release from the soil. Coleman (1973) made the unsupported claim that this condition would be satisfied for an alkali to soil surface area ratio greater than 0.25. Field investigations by Kirita (1971) showed that surface area of the alkali limited the amount of carbon dioxide absorbed when the ratio was less than approximately 0.45. Relatively high (approximately 10 mg CO_2/h) concentrations of carbon dioxide were recorded in Kirita's experiments.

On the basis of these apparently conflicting claims it was considered necessary to carry out an experiment to determine the effect of alkali surface area on the amount of absorbed carbon dioxide.

Respiration chambers were constructed from 1 ℄ 9.0 cm i.d. 'Agee' screw type jars each of which was fitted with a lid held in place by a screw type metal ring to provide an air tight seal. A length of 1/16" gauge steel wire attached at one end to the lid and shaped to form a coil, served to suspend a beaker approximately 5 cm above

the bottom of the jar. The apparatus is shown in Figure 21.

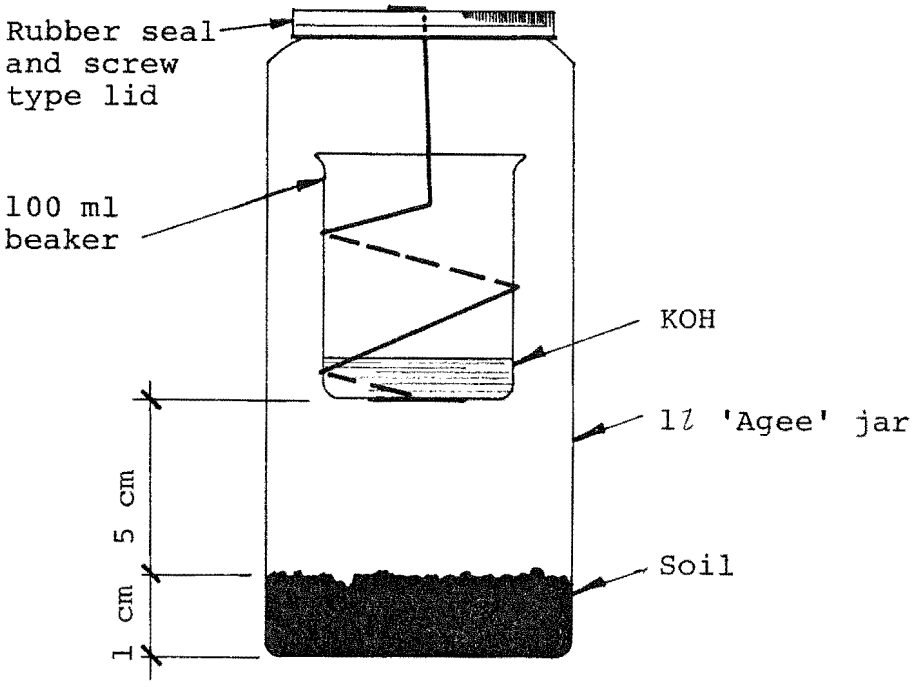
The soil used was taken from a Timaru site adjacent to that used for the oil disposal experiments. A description of the soil is given on p 65 . Approximately 50 kg of top soil (uppermost 15 cm) was removed and transported to the laboratory where it was stored at room temperature until use approximately 3 weeks after collection.

Air-dried 2 mm sieved soil was moistened immediately before the experiment was begun, to provide a moisture content 37 percent of water holding capacity (W.H.C.). This figure was chosen on the basis of preliminary experiments which showed that soil disaggregated at moisture contents greater than 42 percent and as such may have resulted in a lower soil oxygen content due to a decreased soil porosity.

One hundred g of sieved soil on an air-dried soil basis (a.d.s.b.) was added to a respiration chamber and spread evenly over the bottom using a glass rod. The wire coil was fitted with a beaker having an i.d. of 5.0 cm. Two further chambers were similarly prepared but fitted with vessels having internal diameters of 5.6 cm and 6.6 cm respectively. The alkali surface areas as percentages of soil surface areas were 31, 39 and 54 respectively.

A solution of approximately 1N KOH was prepared by dissolving 'Analar' KOH pellets in deionized water. All

Figure 21: Soil respiration chamber



other solutions used for the present and subsequent experiments were similarly prepared unless otherwise stated. Concentration of the KOH solution was determined to ± 0.005 N by titration with HCl which had been standardized to ± 0.005 N using a 0.1 N solution of $Na_2B_4O_7$. Ten ml of KOH solution was added to each alkali vessel using a transfer pipette (Oxford Laboratories, U.S.A.). Each treatment was replicated twice to provide three replicates. The respiration chambers were assembled and incubated in darkness at 19° ± 1.

After 24 h the chambers were opened and the beakers removed. Thirty ml of 0.33 M $BaCl_2$ was added to each beaker of alkali to precipitate any carbonate that may have formed. Two drops of mixed, (1:1 v/v) phenolphthalein/thymolphthalein indicator were added to each vessel and the remaining hydroxyl ions titrated using approximately 1 N hydrochloric acid. The chambers were left open to atmosphere for approximately 1 h to enable the soil to re-equilibrate with the atmosphere. The above procedure was then repeated for an incubation period of 48 h. Subsequent incubations were of 5 and 6 days duration over the experimental period and selected to confirm the pattern of carbon dioxide evolution obtained by other workers for remoistened air-dried soil. The chambers were reweighed prior to every third incubation and deionized water added as required to maintain the soil moisture content which was never less than 97 percent of

the desired level.

Amounts of carbon dioxide absorbed by the alkali were determined using the formula given below:

$$\text{mg } CO_2/100 \text{ g air-dried soil/h} = \left[\frac{\left\{ \left(\frac{\text{N KOH}}{\text{N HCl}} \times 10 \right) - \left(\text{Titrated vol. HCl} \right) \right\} \left[\text{HCl} \right] \times 22}{\text{Incubation time (h)}} \right] 2$$

Results expressed as mg CO_2/100 g air-dried soil/h are given in Figure 22 . Each point on the graph represents the mean rate of carbon dioxide absorbed since the previous sampling. Carbon dioxide totals for the various soils were compared using a one way analysis of variance results of which are shown in Table 51 .

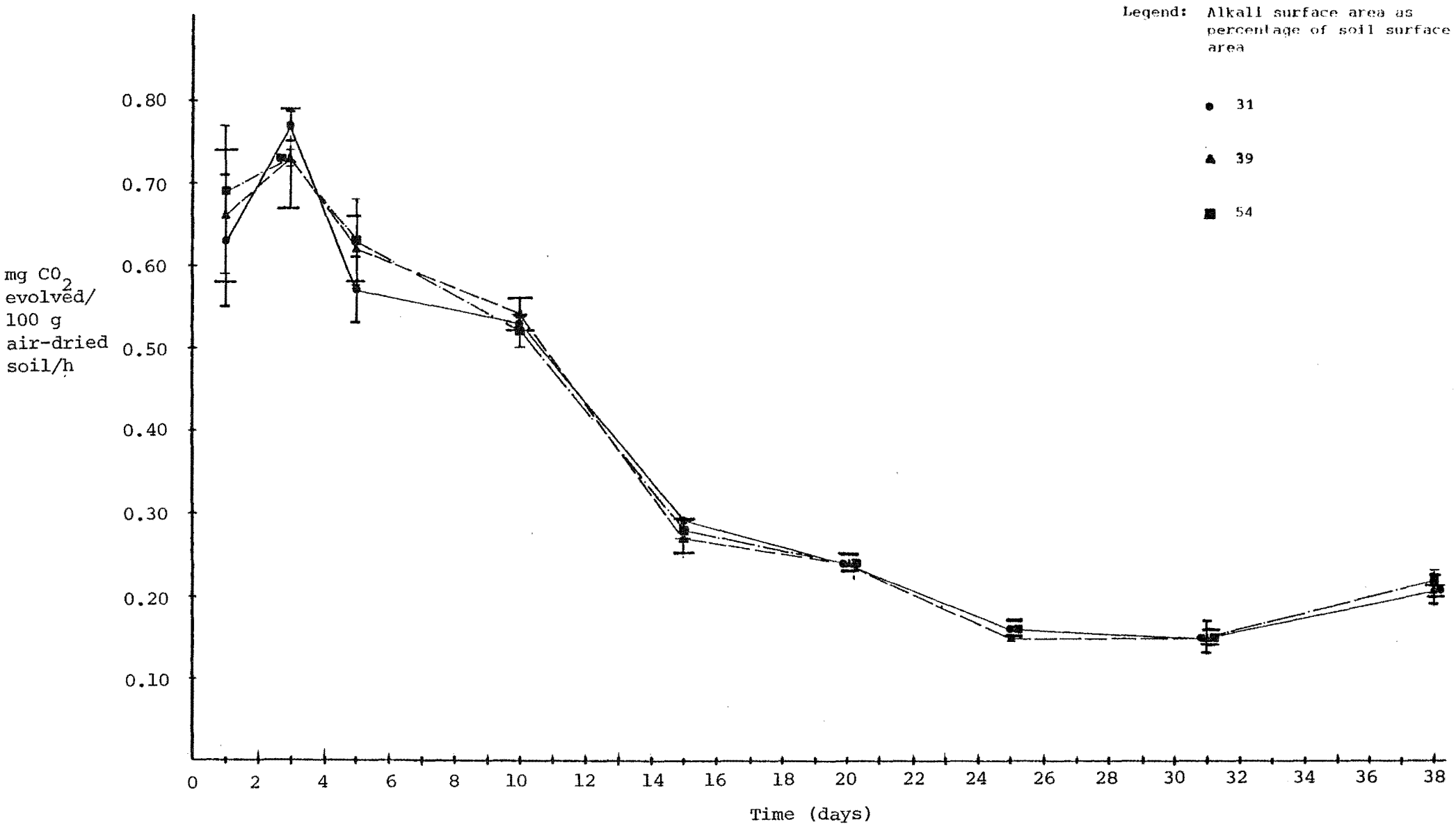
Table 51 : Carbon dioxide absorbed by alkali traps of varying surface area. + Means of 3 replicates.

	Alkali surface area as percentage of soil surface area		
	31	39	54
Weight CO_2 absorbed over 38 d mg +	277.8	276.8	288.4

$F_{(2,6)} = 0.348$ n.s.

There was no significant difference in the amounts of carbon dioxide measured for the three treatments and it was therefore concluded that the amount of carbon dioxide absorbed by the alkali was not affected by the alkali

Figure 22: Effect of alkali surface area on carbon dioxide absorption.



surface areas tested, a conclusion which was in agreement with the claim of Coleman (1973). For convenience, 100 ml beakers were used for all future respiration experiments.

The data indicate an initial burst of activity followed by a progressive decline to an average value of approximately 0.2 mg/100 g soil/h after 25 d. A similar pattern of carbon dioxide evolution following remoistening of air-dried soil has been observed by many workers for a wide variety of soils, (Stevenson 1956, Soulides and Allison 1961, Ino and Monsi 1969 and Ross and Cairns 1978). Several explanations have been advanced to explain the activity.

Air-drying of soils was shown by Lebedjantzev (1924) to result in several chemical changes in the soil. These included small changes in the solubility of mineral substances, large increases in the solubility of organic substances, large increases in nitrogen and phosphorus, large increases in NH_3-N and amide nitrogen and a sharp decrease in numbers of micro-organisms. Soulides and Allison (1961) attributed the stimulated respiratory activity following remoistening of soil to the use of readily available nutrients by the 'youthful' state of a rapidly growing bacterial population.

(b) Evolution of Carbon Dioxide from Biologically Inactive Timaru Silt Loam. Carbon dioxide produced by a soil may result from nonbiological as well as biological processes. Sources of nonbiological carbon

dioxide have been reviewed by Stotsky (1965) and include that produced by cell-free heat stable enzymes and the action of added chemicals on free soil carbonates or organic acids produced during metabolism. Nonbiologically produced carbon dioxide if produced in significant amounts would possibly interfere with measurements of soil respiration. In order to measure soil respiration it was therefore necessary to have a method by which soil could be rapidly rendered sterile or alternatively, biologically inactive. The aim of the following experiment was therefore two-fold: to determine the relative levels of biological and nonbiological soil carbon dioxide production and to investigate methods by which soil could be rendered biologically inactive.

Methods of sterilization have been described by Pelczar et al. (1977) and reviewed by Parkinson et al. (1971) who grouped them according to whether heat, chemicals or gamma radiation is used to treat the soil. All were considered by Parkinson et al. to chemically modify the soil to varying extents.

Steam sterilization, although a very effective and commonly used method was not convenient for the present investigation because of the large number of samples which would require treatment each time the soil was exposed to the atmosphere for carbon dioxide determination.

Treatment of the soil with enzyme inhibiting metal salts added in solution (Pelczar et al. 1977) presented a

possible alternative method. Provided that the salts remained toxic only one application would be needed.

A 1:1 mixture of saturated solutions of copper sulphate and mercuric chloride was used by Vossbrinck et al. (1979) to inhibit biological activity in small samples of blue gamma litter. One hundred ml of solution was applied monthly to 3 g samples contained in 53 μ m mesh bags. The samples were exposed to atmosphere and in contact with the soil surface between applications. Populations of bacteria, actinomycetes and fungi recorded in monthly plate counts were lower by a factor of 10^3 compared with those of untreated litter. No growth was recorded for several samples of treated soil.

Air-dried, 2 mm sieved Timaru soil was moistened with deionized water to provide a moisture content 37 percent of W.H.C. One hundred g of soil (a.s.d.b.) was weighed into each of three respiration chambers and spread evenly over the bottom with a glass rod. A saturated solution of $HgCl_2$ was prepared and added to an equal volume of saturated $CuSO_4$ solution. Sufficient $HgCl_2/CuSO_4$ solution was then mixed thoroughly with a further 100 g of Timaru soil to provide a water content 37 of W.H.C. Water amended and $HgCl_2/CuSO_4$ treated soils were replicated 5 and 2 times respectively. Three further chambers without soil served as controls.

Three chambers containing water amended soil were autoclaved at 121°C for 1 h and allowed to cool to ambient

temperature in a sterile cabinet. Other experimental details were as for the previous experiments except that KOH was added aseptically to the autoclaved soil chamber and the strength of solution reduced for the treated soils to approximately 0.2 N in anticipation of reduced carbon dioxide production.

Mean carbon dioxide totals for the various soils and control were compared using a one way analysis of variance. Results shown in Table 52 indicate that totals for autoclaved and chemically treated soil were not significantly different.

Table 52 : Evolution of carbon dioxide by biologically inactive Timaru silt loam. + Means of 3 replicates. Incubation temperature, 19°C.

	Soil	Soil + $HgCl_2$/ $CuSO_4$	Autoclaved Soil	Control- no soil
Total CO_2 evolved/100 g soil over 33d (mg) +	402.48	43.27	48.31	4.77

$F_{(3,8)}$ = 388.8 *** SE = ± 5.7849 L.S.D. 5% = 21.40

It was therefore concluded that the combination of $HgCl_2$ and $CuSO_4$ effectively rendered the soil biologically inactive. After correction for carbon dioxide absorbed from the atmosphere, production from the treated soils was approximately 10 percent of that obtained for untreated soil. An air volume of 950 ml was assumed for

chambers containing soil. Average atmospheric carbon dioxide concentration was 310 µg/g.

Mean average carbon dioxide totals for each incubation period are shown in Figure 23 . Average production from the treated soils over 33 d did not exceed 14 percent of the untreated soil after correction for atmospheric carbon dioxide.

Hydrocarbons may also be decomposed by both biological and nonbiological processes, (p5). As part of the present study it was proposed to investigate possible changes in the amount and chemical composition of oil added to Timaru soil and to apportion any such changes to biological and nonbiological causes.

A further experiment was therefore carried out to compare the effect of mercury and copper salts on the amount of carbon dioxide evolved by Timaru soil with and without oil.

Soil treated with $HgCl_2$ and $CuSO_4$ was prepared as for the previous experiment. A further sample was similarly prepared except that 'Vitrea 22' oil was added at a rate of 5 percent by weight (a.d.s.b.) and mixed thoroughly with the moistened soil. A respiration chamber containing no soil served as a control. Treatment and control soils were replicated twice.

Results given in Table 53 and Figure 23 show that a combination of $HgCl_2$ and $CuSO_4$ effectively inhibited

Figure 23: Effect of autoclaving and metal salts on evolution of carbon dioxide by Timaru silt loam incubated at 19°C.

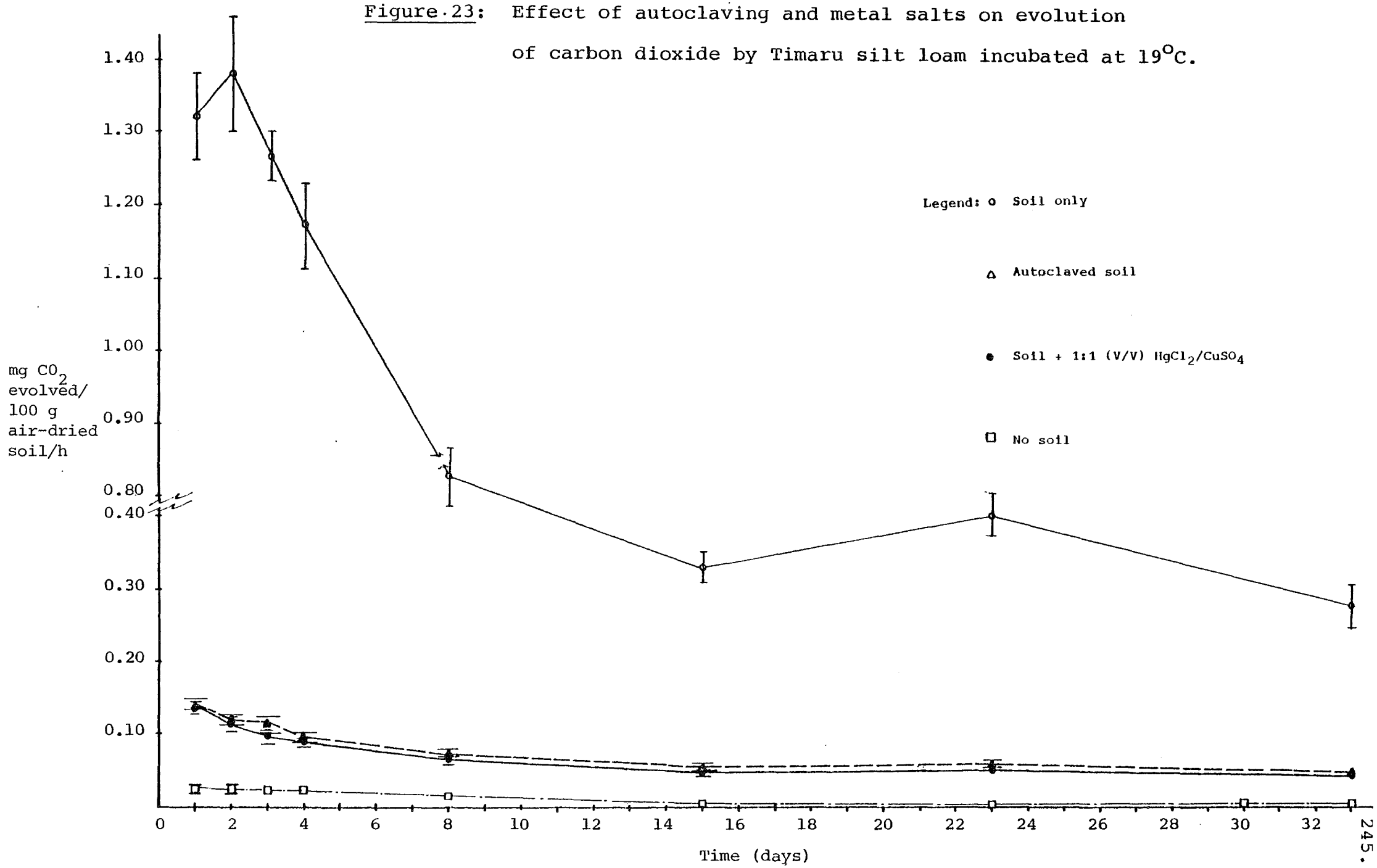


Table 53 : Effect of 'Vitrea 22' oil on evolution of carbon dioxide by biologically inactive Timaru silt loam. +Means of 3 replicates. Incubation temperature, 19°C.

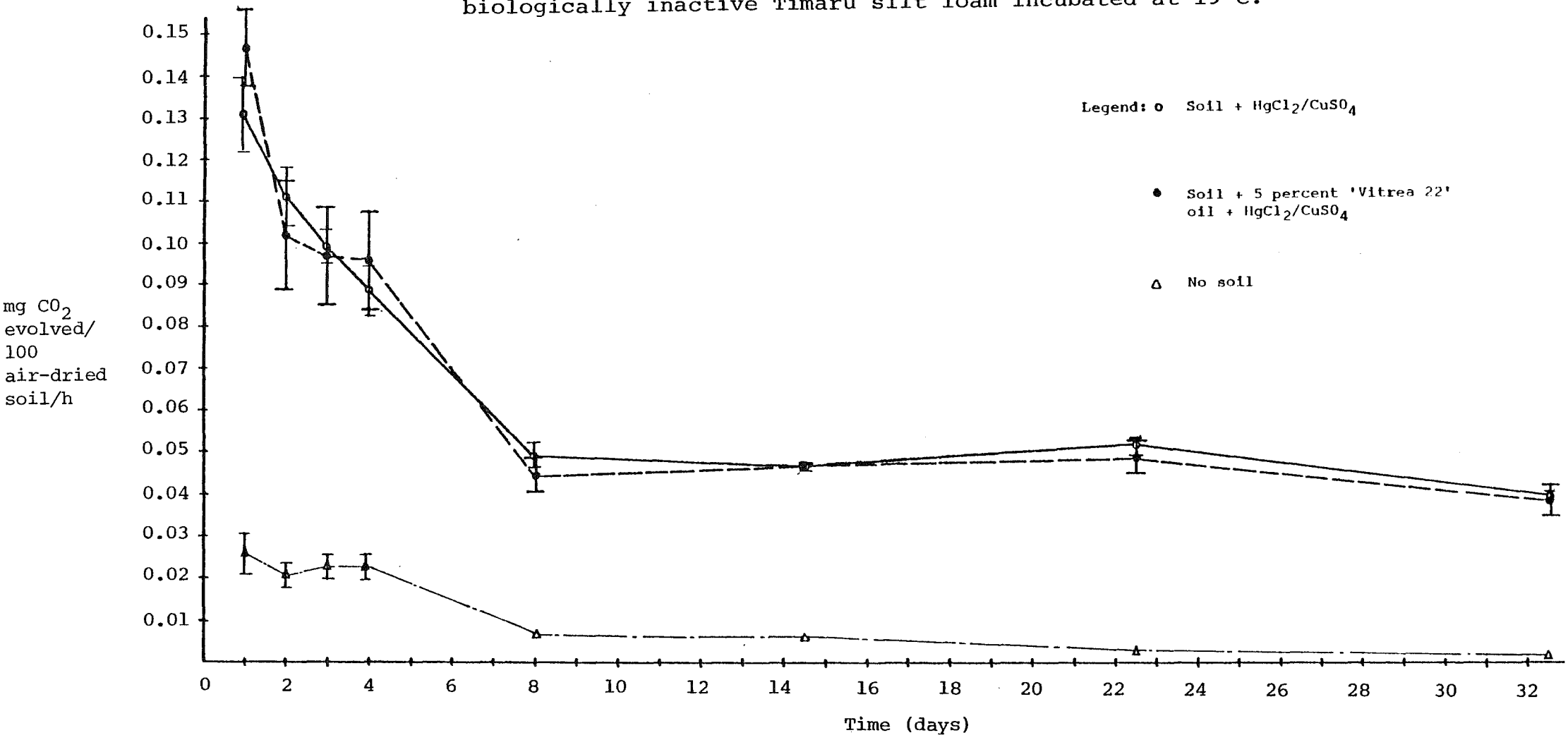
	Soil + $HgCl_2$/ $CuSO_4$	Soil + 5% 'Vitrea 22' oil + $HgCl_2$/ $CuSO_4$	Control- no soil
Total CO_2 evolved/ 100 g air-dried soil over 33 d (mg)+	42.53	41.24	4.18

$F_{(2,6)}$ = 973.80** S.E. = ± 0.4935 L.S.D.(5%) = 1.75

biological activity of the oil amended soil and that non-biological production of carbon dioxide was not increased by the addition of oil. Together the experiments indicated that addition of metal salts to soil is a satisfactory alternative method by which biological activity of soil may be suppressed.

(c) The effect of Soil Moisture Content and Soil Depth on the Rate of Carbon Dioxide Released from Timaru Silt Loam. For a given soil, air supply and moisture content influence the extent and rate of assimilation of carbonaceous substrates and hence carbon dioxide production, (Alexander 1971). Whilst carbon dioxide is released from completely anaerobic systems, oxygen tends to stimulate carbon mineralization. Water is considered to be limiting when its addition to soil stimulates carbon dioxide production. Conversely,

Figure 24: Effect of 'Vitrea 22' oil on evolution of carbon dioxide by biologically inactive Timaru silt loam incubated at 19°C.



excessive soil moisture may result in a diminished level of production. This was considered by Alexander (1971) to be due to a reduction in microbial activity resulting not from the direct effect of water but from the indirect result of moisture on the movement of oxygen through soil.

Katznelson et al. (1956) found that oxygen uptake by soil treated with a mixture of amino acids was greatest for moisture contents of 60-80 percent of water holding capacity. In another study, Ino and Monsi (1969) investigated the effect of soil moisture content on carbon dioxide released from soils of varying texture and soil organic matter content. Maximum evolution rates were recorded for moisture contents ranging from 51-89 percent of water holding capacity depending on soil type and temperature. The effect of soil depth on the rate of carbon dioxide evolution was also studied. Activity of an allophane soil was considered to be reduced for soil depths of approximately 3.5 cm and greater. No indication of the variability of the data was given but the results presented suggest that soil depths of greater than 1 cm may have resulted in reduced rates of carbon dioxide production.

Where soil carbon dioxide is limited by soil moisture, depth of both it is conceivable that any potential stimulation due to the addition of a carbonaceous substrate would be masked. An experiment was therefore carried out to investigate the effect of soil moisture content and soil

depth on the evolution of carbon dioxide Timaru soil.

Sieved (2 mm) Timaru silt loam was moistened with deionized water to 37 percent of W.H.C. Aliquots 50 g, 100 g or 150 g of soil (a.d.s.b.) were weighed into each of three respiration chambers. The soil was spread evenly over the bottom of each chamber to give soil depths of approximately 1, 2 and 3 cm respectively. The procedure was repeated twice using the same soil except that soil moisture contents were 52 and 67 percent of water holding capacity respectively. Results obtained for the previous experiment indicated that carbon dioxide produced by nonbiological processes was relatively small and it was therefore considered unnecessary to distinguish it from biologically produced carbon dioxide. Sterile controls were therefore omitted. Each treatment was replicated twice (3 replicates). Other experimental details were as described for experiment 'a'.

Mean average amount of carbon dioxide evolved during each incubation were determined for all treatments and are shown in Figures 25-27. Activity of all treatments approximated an exponential function with respect to time. The rate of change in $C0_2$ production after 57 d was considered sufficiently small to permit a comparison of treatments. Carbon dioxide evolution rates after both 57 d and 74 d were compared using a two-way analysis of variance. Results given in Tables 54-56 indicate that carbon dioxide production was consistently highest for the

Table 54: Effect of soil depth and moisture content on evolution of carbon dioxide by Timaru silt loam after 57 d incubation. Analysis of variance table.

Source		d.f.	F
Soil depth	D	2	572.61 ***
Soil moisture content	M	2	66.99 ***
	DM	4	21.08 ***

Table 55: Effect of soil depth and moisture content on evolution of carbon dioxide by Timaru silt loam after 74 d incubation. Analysis of variance table.

Source		d.f.	F
Soil depth	D	2	183.35 ***
Soil moisture content	M	2	153.76 ***
	DM	4	16.90 ***

shallowest (1 cm) soils irrespective of moisture content and lowest for 3 cm deep soils.

The soil used had a very poorly developed structure and it was therefore likely that the average amount of oxygen available to soil micro-organisms was less for the deeper soils. The results also provide evidence that a soil moisture content of 37 percent was less than optimum for carbon dioxide production. Presumably disaggregation of the soil was not sufficient to have adversely affected the passage of oxygen through the soil. Production was significantly less for soils having a water content 37 percent of water holding capacity. Activity was not significantly different for soils having water contents 52 and 67 percent of water holding capacity suggesting that the soil moisture content was within the range which was optimum for carbon dioxide production.

Table 56: Effect of soil moisture and soil depth on evolution of carbon dioxide by Timaru silt loam. +Means of 3 replicates. Incubation temperature, 19°C.

	Soil depth (cm)	Incubation interval	Soil moisture content as % of W.H.C.		
			37	52	67
Mean weight CO_2 evolved 100 air-dried soil/h (mg)+	1	46d - 57d	0.26	0.33	0.31
		57d - 74d	0.21	0.27	0.33
	2	47d - 57d	0.20	0.20	0.23
		57d - 74d	0.17	0.21	0.22
	3	46d - 57d	0.19	0.22	0.22
		57d - 74d	0.15	0.19	0.22

S.E. (46d - 57d = ± 0.001015 L.S.D. (5%) = 0.006

S.E. (57d - 74d = ± 0.000107 L.S.D. (5%) = 0.003

Figure 25: Effect of soil moisture on evolution of carbon dioxide by Timaru silt loam of 1 cm depth. Incubation temperature = 19°C.

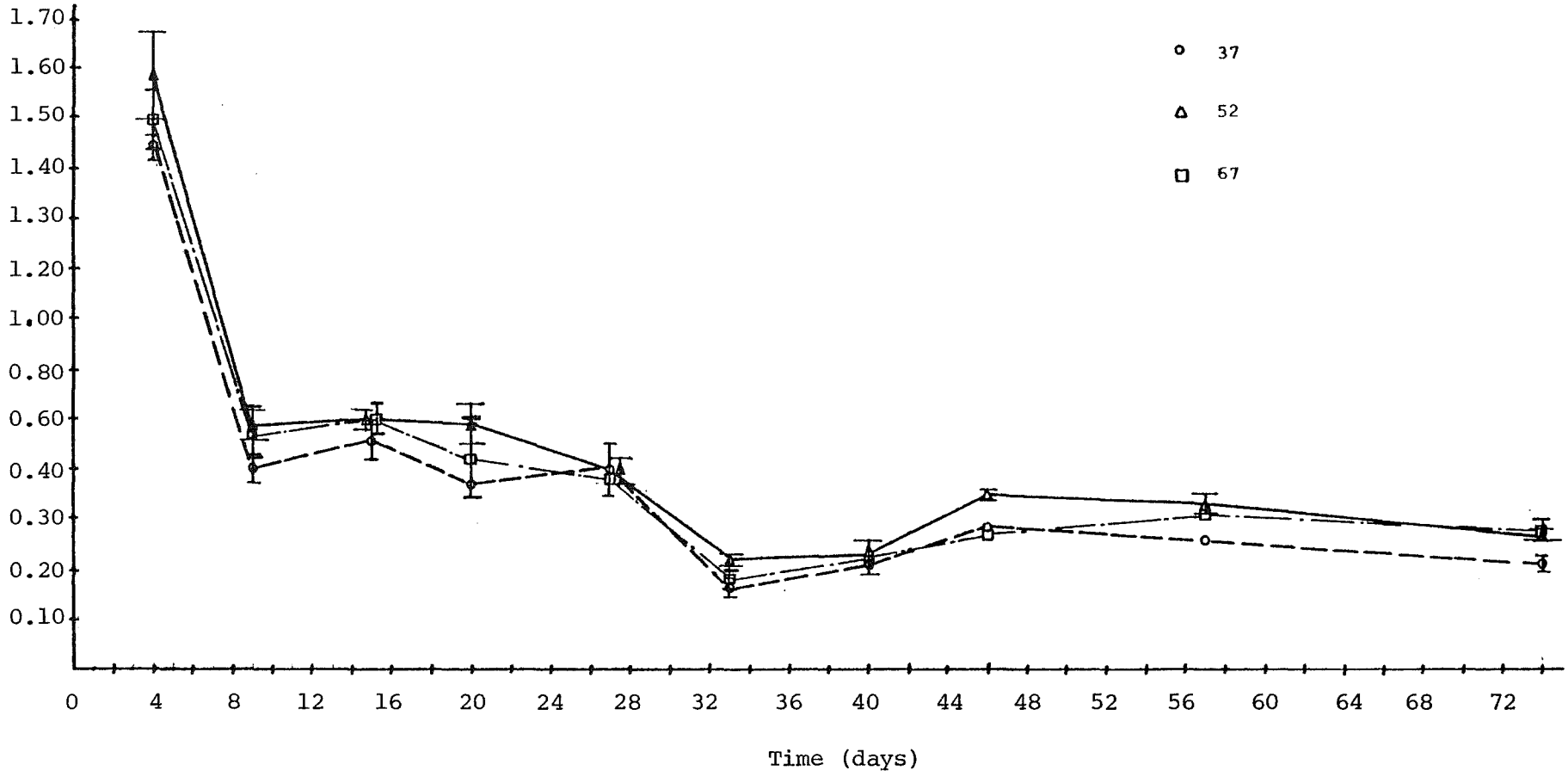


Figure 26: Effect of soil moisture on evolution of carbon dioxide by Timaru silt loam of 2 cm depth. Incubation temperature = 19°C.

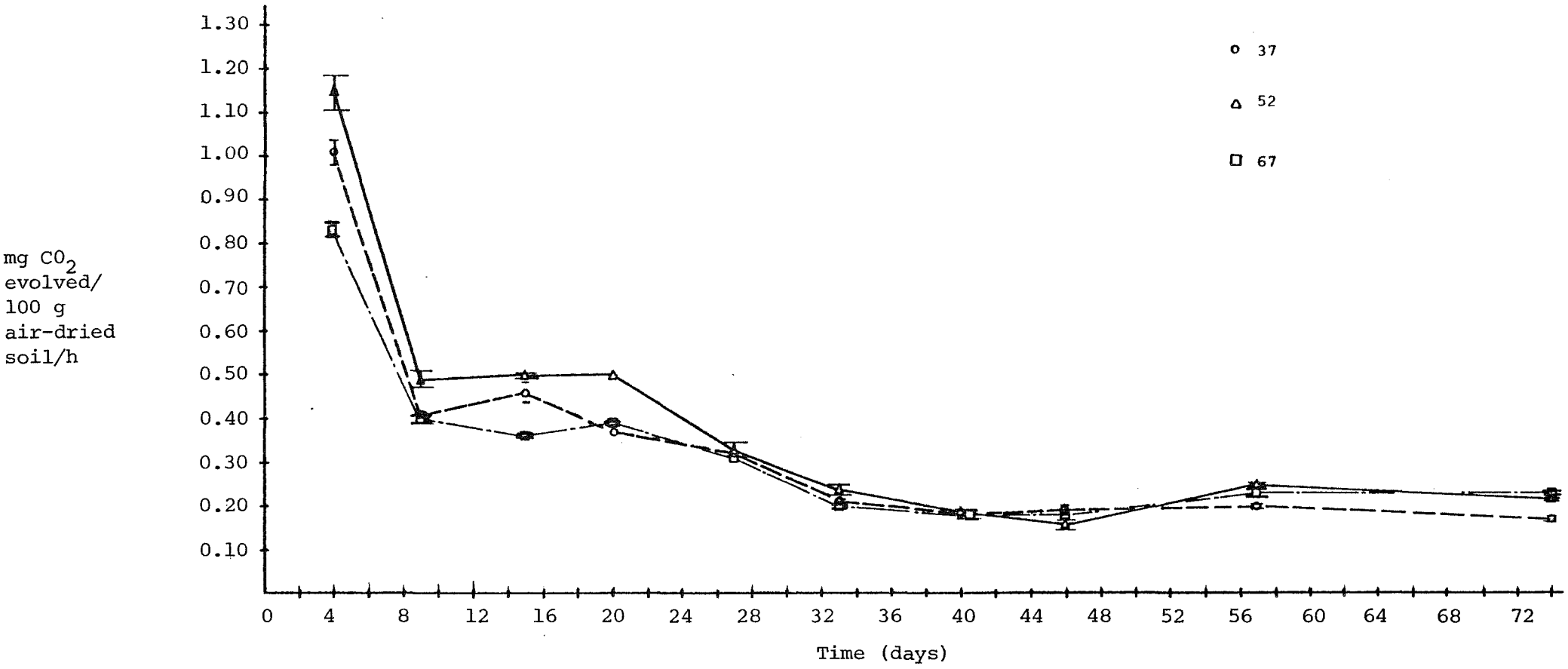
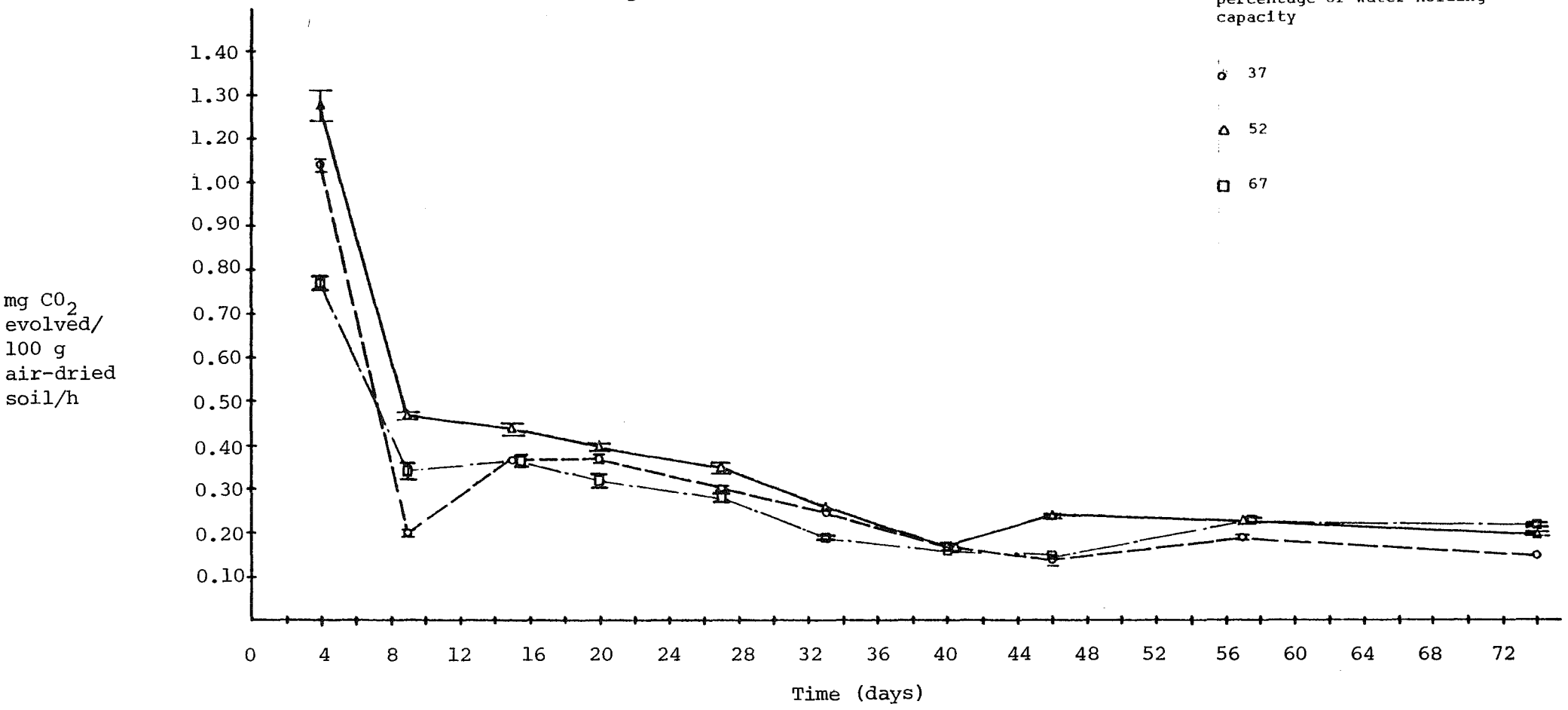


Figure 27: Effect of soil moisture on evolution of carbon dioxide by Timaru silt loam of 3 cm depth. Incubation temperature = 19°C.



'Stable' production values obtained for a soil depth of 1 cm and moisture contents of 52 and 67 percent were similar to those obtained by Ross and Cairns (1978) for 40 g samples of Tawhiti silt loam moistened to 60 percent of water holding capacity and incubated at 20^{o}C in Biometer flasks.

On the basis of results obtained for the present investigation, a soil depth of 1 cm and water content 52 percent of water holding capacity were used for all future experiments including controls.

III. THE EFFECT OF 'VITREA 22' OIL ON EVOLUTION OF CARBON DIOXIDE BY TIMARU SILT LOAM

One purpose of the present investigation was to determine whether hydrocarbons could be decomposed in soil by micro-organisms. Waste lubricating oil applied to soil at Timaru, because it contained not only a complex mixture of hydrocarbons but also other potential microbial substrates such as detergents was not a suitable source of pure hydrocarbon. Instead, 'Vitrea 22', a paraffinic lubricating oil free of additives (p139) was used.

Sieved (2 mm) air-dried Timaru soil was moistened to 52 percent of W.H.C. and mixed thoroughly with 'Vitrea 22' oil added at 5 percent by weight (a.d.s.b.). Fifty g of soil (a.d.s.b.) was weighed into a respiration chamber and spread evenly over the bottom. A second chamber contained only moistened soil and a third which contained soil amended with $HgCl_2$ and $CuSO_4$ as described for the previous experiment served as a control.[+] pH of the oil amended and unamended soils was 6.45 and 7.00 respectively.

Acidity of the 'Vitrea 22' oil was negligible, (Total acid no. 3.5×10^{-3} mg KOH/g - Shell Oil N.Z. Ltd. pers. comm. 1980) and the pH of the oil amended soils was therefore presumably masked by the oil. For this reason and because the soil used had previously been limed for the field experiment, pH of the oil treated soils was not adjusted. Other experimental details were as for previous experiments except that the concentration of KOH and HCl used was increased to approximately 1.6 N in anticipation of increased levels of activity.

[+] Moisture content 52 percent of W.H.C.

Rates and amounts of carbon dioxide evolved by the soils were computed over the experimental period of 38 d and are shown in Table 57 and Figure 28 . The plotted points in Figure 28 each indicate the mean amount of carbon dioxide evolved since the previous determination. Assuming a chamber air volume of 950 ml, maximum percentage oxygen removed from the atmosphere during a single incubation was generally less than 30 percent. Results indicate that carbon dioxide produced by Timaru soil was increased by 210 percent in the presence of 'Vitrea 22' oil. Increased activity followed an initial 'lag phase' of 1-2 d during which CO_2 production was less or only slightly greater than that for unamended soil. As discussed elsewhere, (p229), the 'lag phase' may have resulted from the need for the microbial population to adapt to provide the enzymes needed to decompose the oil.

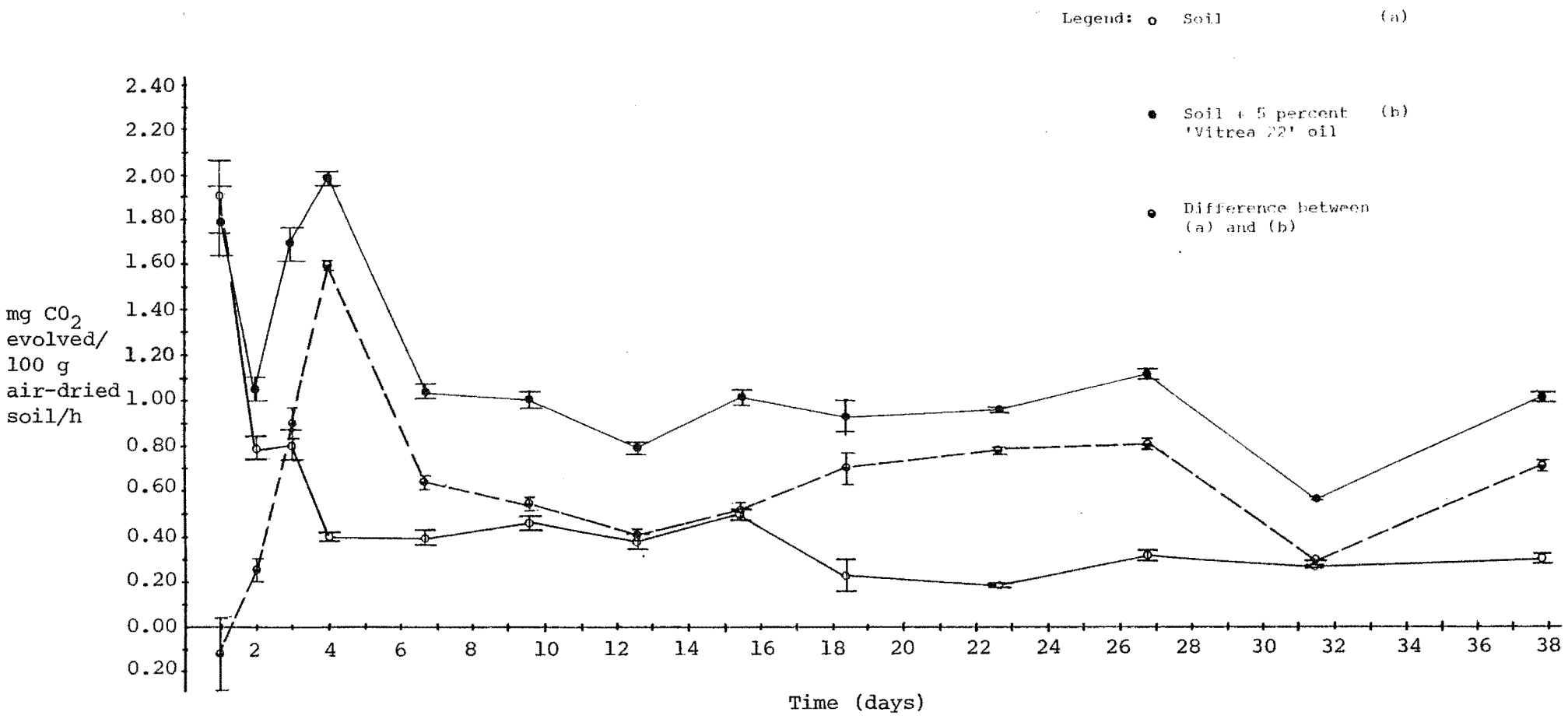
Table 57 : Effect of 'Vitrea 22' oil on evolution of carbon dioxide by Timaru silt loam.

$^+$ Means of 3 replicates. Incubation temperature, 19°C.

	Soil + 5% oil	Soil	Soil + $HgCl_2$/ $CuSO_4$
Total CO_2 evolved/ 100g air-dried soil over 38d (mg)$^+$	882.31	338.18	45.96
Equivalent weight of carbon evolved/ 100g air-dried soil over 38d (mg)	240.87	19.32	12.44

S.E. = ± 17.32 L.S.D. (5%) = 61.39

Figure 28: Effect of 'Vitrea 22' oil on evolution of carbon dioxide by Timaru silt loam incubated at 19°C.



The difference in total carbon evolved by oil treated and unamended soils over 38d was 148.55 mg. Assuming a general formula of Cn H_{2n} for the oil then the difference is equivalent to: 148.55 x 1.17 or 173.80 mg mineralized oil or an average rate of mineralization of 3.5 percent/ month. For reasons given on p228 the rate was considered a maximum.

IV. EFFECT OF OIL APPLICATION RATE, REAPPLICATION AND NUTRIENTS ON CARBON DIOXIDE EVOLVED BY TIMARU SILT LOAM

As considered elsewhere, oil, when it is first added to soil is likely to be decomposed relatively slowly due to the need for the microflora to adapt to provide the necessary enzymes to decompose the various hydrocarbons present. The initial respiratory 'lag phase' observed for oil amended soil in the previous experiment was evidence for such a need. Oil was applied to the Timaru field plots only once but practical application of a land disposal system would require several applications. With further addition it was conceivable that oil would be decomposed more rapidly by an adapted microbial population.

Rate of disappearance of waste lubricating oil from Timaru soil under field conditions increased with application rate. Negligible contamination of subsoil and runoff water suggested that the increase was due to some factor other than leaching.

An increase in the average rate of oil disappearance from Timaru soil under field conditions following the

application of nitrogen and the lower exchangeable NO_3, NO_2 and NH_3 content of the oiled soils at the conclusion of the experiment were evidence that nitrogen was used by soil micro-organisms. Evidence for its immobilization in the oiled soils suggested that phosphorus was also used by micro-organisms.

The following experiment was therefore carried out to investigate the possible effects of oil application rate, reapplication and nutrients on the evolution of carbon dioxide by Timaru soil.

Fifty g of air-dried 2 mm sieved Timaru soil was moistened to 52 percent of W.H.C. and placed in a respiration chamber. A nutrient solution containing nitrogen, phosphorus and potassium was prepared by dissolving ammonium nitrate, (1.09 g/ℓ) and potassium di-hydrogen orthophosphate, (507 mg/ℓ) in deionized water. Sufficient solution to provide a soil moisture content 50 percent of W.H.C. was mixed thoroughly with a further 50 g of soil and placed in a second respiration chamber. Soil concentrations of the added nutrients were: N (160 µg/g; P (52 µg/g) and K (60 µg/g). One further chamber containing nutrient amended soil and another, only moistened soil were similarly prepared except that 'Vitrea 22' oil was mixed with the soil at a rate of 5 percent by weight (a.d.s.b.). The procedure was repeated for an oil application rate of 10 percent. Fifty g of soil treated with $HgCl_2$ and $CuSO_4$ served as a control. pH of the soils

amended with oil ranged from 6.25 to 6.45; that for unamended soils 6.90 to 7.00. For reasons given on p 256 pH of the oil treated soils was not adjusted.

Treatment and control soils were each replicated twice. Three further treatment replicates were prepared for oil reapplication. Nine further replicates each of soil + nutrients, soil + nutrients + 5 percent oil and soil + nutrients + $HgCl_2/CuSO_4$ solution were prepared for extraction and gas chromatographic analysis. To permit a distinction between biological and possible nonbiological oil decomposition, nine replicates comprising soil + nutrients + oil + $HgCl_2/CuSO_4$ solution were also included. Carbon dioxide was not determined for replicates prepared for extraction. Oil was reapplied after 66 d at the same rates to 3 replicates of each oil treated oil. The oil was weighed into the respiration chambers and mixed thoroughly with the soil using a glass rod. Samples which received no further oil were otherwise similarly treated. All chambers were left open for 2 h to enable the soils to re-equilibrate with the atmosphere. Other experimental details were as described for experiment 'a'. Time did not permit continuation of the experiment until carbon dioxide production rates for all soils had restabilized and it was therefore terminated after 106 d.

Total carbon dioxide evolved from 0 d to 66 d and 67 d to 106 d was computed for treatment and control soils and compared using 2 & 3 way analyses of variance. Results

are given in Tables 58-60. The difference between the amount of carbon dioxide evolved by each oil amended soil and that produced by the appropriate control was also determined. Results are shown in Figures 29 and 30. Each point on the graph represents the mean rate of carbon dioxide evolution for the period elapsed since the previous determination. Results (67 d - 106 d) for soils which received a single oil application are shown in both figures to permit a visual comparison with those obtained for reoiled soil. Maximum amounts of oil mineralized during the period 0 d to 66 d and 67 d to 106 d were determined using assumptions given on p258. Results are shown in Table 61. Oxygen removed from the atmosphere during any one incubation was generally less than 45 percent.

Carbon dioxide evolution during the first 66 d of the experiment was increased by the addition of oil at both rates but was greater for oil added at the 10 percent application rate. Production from both unoiled and oiled soils was increased by the addition of nutrients.+ The greatest increase, 51 percent, was obtained for an oil concentration of 5 percent; the smallest, 4 percent, obtained for unoiled soil, was not significant.

The pattern of carbon dioxide evolution to 66 d was similar to that obtained for the previous experiment. Production from the oil treated soils was initially reduced or only slightly greater than that determined for the unoiled control soil. Subsequently, much greater

+ Data for nutrient amended unoiled soils showed the same pattern and similar rates of CO_2 production and were omitted from Figure 29 for clarity.

Table 58: Effect of oil application rate, oil reapplication and nutrients on evolution of carbon dioxide by Timaru silt loam. 0-d - 66 d incubation. Analysis of variance table.

Source		d.f.	F
Oil application rate	0	1	1326.63 ***
Nutrients	N	1	704.77 ***
	0.N	1	163.07 ***

Table 58a: 67 d - 106 d incubation. Analysis of variance table.

Source		d.f.	F
Oil application rate	0	1	83.98 ***
Nutrients	N	1	191.95 ***
Oil reapplication	R	1	22.15 ***
	0.N	1	17.93 ***
	N.R	1	0.4 n.s.
	0.R	1	19.93 ***
	0.N.R	1	29.01 ***

Table 59 : Effect of oil application rate, oil reapplication and nutrients on evolution of carbon dioxide by Timaru silt loam incubated at 19°C. +Means of 3 replicates. NR = No reapplication of oil. R = reapplication of oil at same rate.

	Treatment									Control		
	Incubation period (d)	Soil + 5% 'Vitrea 22' oil		Soil + 5% 'Vitrea 22' oil + nutrients		Soil + 10% 'Vitrea 22' oil + nutrients		Soil + 10% 'Vitrea 22' oil + nutrients		Soil	Soil + nutrients	Soil $HgCl_2$/ $CuSO_4$
		NR	R	NR	R	NR	R	NR	R			
Total CO_2 evolved /100g air-dried soil (mg)+	0-66	1427.26	1427.26	2256.26	2256.26	1920.29	1920.29	2610.10	2610.00	496.82	517.60	72.12
	67-106	1674.77	1854.93	2335.13	2092.32	1929.56	2124.41	2652.51	3441.85	248.25	258.94	37.41
Total carbon evolved as carbon dioxide /100g air-dried soil (mg)+	0-66	389.64	389.64	615.96	615.96	524.24	524.24	712.56	712.56	135.63	141.30	19.69
	67-106	457.21	506.40	637.49	571.20	526.77	579.96	724.14	939.63	67.77	70.69	10.21

S.E. 0-66 d = ± 12.5654 L.S.D. (5%) = 46.27

S.E. 67-106 d = ± 32.9180 L.S.D. (5%) = 132.00

Table 60: Effect of oil application rate, oil reapplication and nutrients on rate of oil mineralization in Timaru silt loam. +Means of 3 replicates. N.R. = No reapplication of oil. R = Reapplication of oil. Incubation temperature, 19°C.

		Treatment							
	Incubation period (d)	Soil + 5% 'Vitrea 22' oil		Soil + 5% 'Vitrea 22' oil + nutrients		Soil + 10% 'Vitrea 22' oil		Soil + 10% 'Vitrea 22' oil + nutrients	
		NR	R	NR	R	NR	R	NR	R
Maximum weight oil mineralized/ 100g air-dried soil (mg)+	0-66	297.19	297.19	555.35	555.35	454.67	454.67	668.37	668.37
	67-106	455.64	513.20	663.16	585.60	537.03	599.26	764.52	1016.65
Maximum weight oil mineralized as % of oil added	0-66	5.94	5.94	11.11	11.11	4.55	4.55	6.68	6.68
	67-106	9.11	5.13	13.26	5.86	5.38	3.00	7.65	5.08
Maximum average rate of oil mineralization mg oil/100g air-dried soil/month	0-66	135.09	135.09	252.43	252.43	206.67	206.67	303.80	303.80
	67-106	350.49	394.77	510.12	450.46	413.10	460.97	588.09	782.04

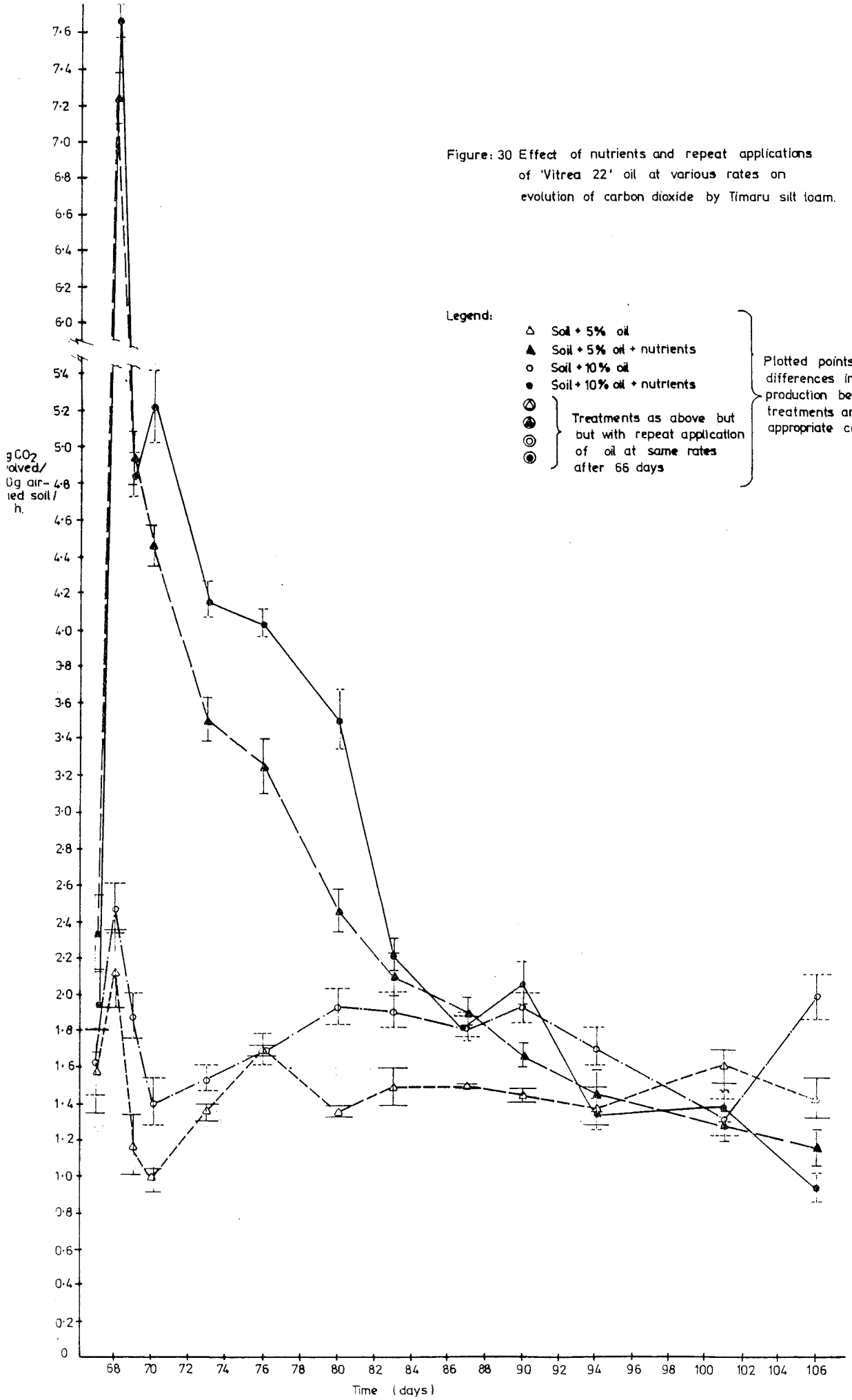


Figure: 30 Effect of nutrients and repeat applications of 'Vitrea 22' oil at various rates on evolution of carbon dioxide by Timaru silt loam.

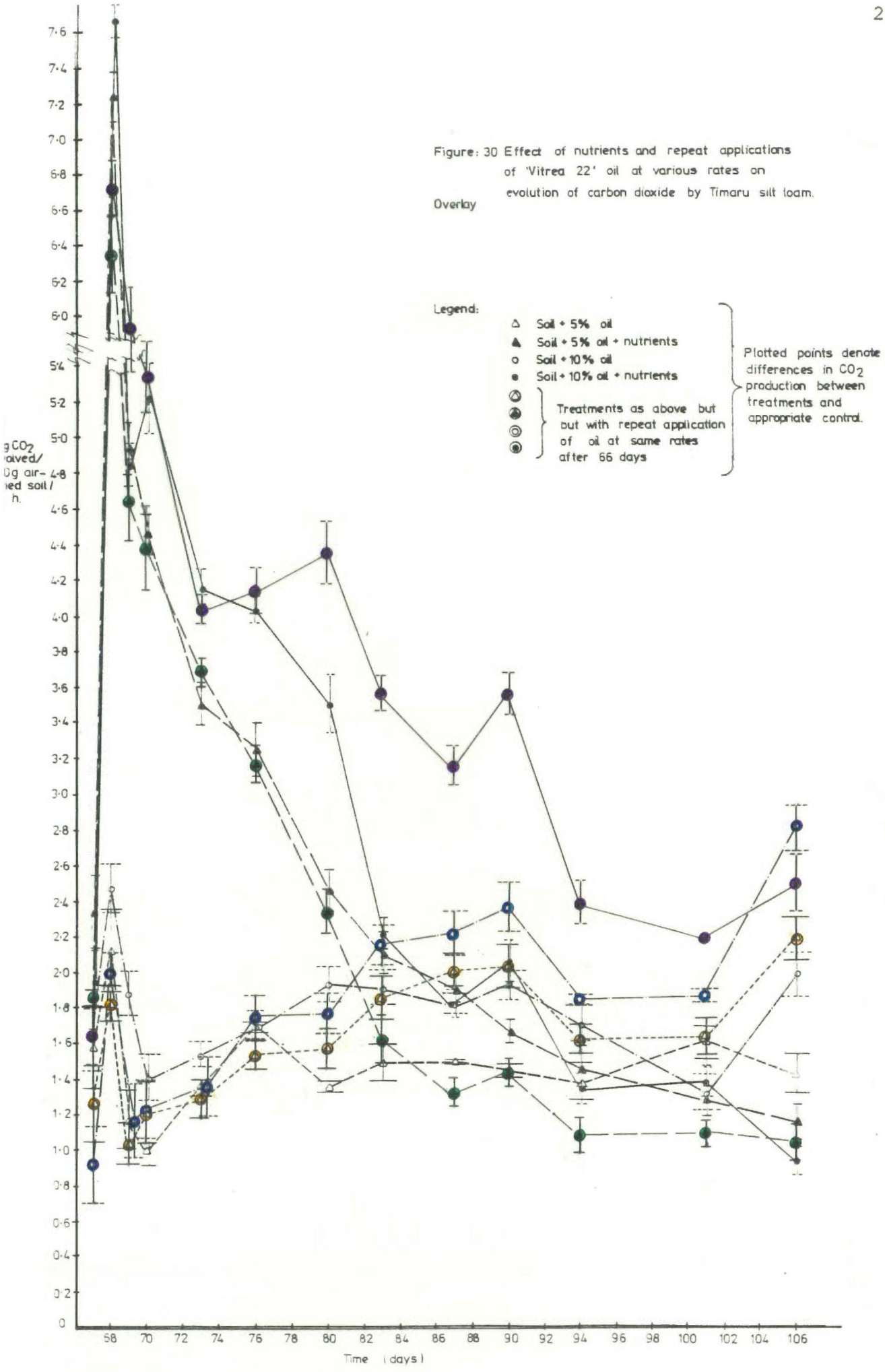


Figure: 30 Effect of nutrients and repeat applications of 'Vitrea 22' oil at various rates on evolution of carbon dioxide by Timaru silt loam.

Overlay

production was obtained for the oil amended soils. Whilst the greatest amount of oil mineralized over 66 d, 668.37 mg/100g soil was obtained for soil containing 10 percent oil and nutrients, maximum average monthly rate of oil mineralization for the same period expressed as a percentage of that added was obtained for the soil amended with 5 percent oil and nutrients.

In contrast to the lag phase which followed the initial application of oil, carbon dioxide production increased immediately after reapplication to all oil treated soils. An initial increase was also obtained for soils which received no further application and for unoiled soils. The magnitude of the increase was several times greater for nutrient amended oiled soils irrespective of the number of oil applications. The increase for these soils was also much greater than that obtained during the first part of the experiment. That obtained for oiled soil without added nutrients was approximately the same and that for unoiled soils, considerably less.

Although production from the oiled soils had not stabilized after 106 d some trends were apparent. Relative amounts of carbon dioxide obtained for soil which received a single application of oil and for unoiled control soils during the period 67 d to 106 d were the same as those obtained for the first part of the experiment. The pattern was repeated for soils which received a second oil application. Production from oiled soils at both application

rates was consistently higher during the second part of the experiment. That for soil containing added nutrients and a single 10 percent application of oil, although initially higher during the same period, declined to smaller values than that obtained after the first 40 d for the same treatment. Production from soils which received a second application of oil was, with one exception consistently higher than that for soils which received a single application. The increase was greatest for soil treated with 10 percent oil and nutrients.

The pattern of results obtained for the second part of the experiment (67 d - 106 d) indicated that at least part of the initial increase in carbon dioxide production resulted from disturbance of the soil at the time of oil reapplication. Remixing of the soil may have increased the amount of oil accessable to soil micro-organisms. Under such conditions and assuming other factors were not limiting, oil decomposition would likely have proceeded at a greater rate. Part of the observed increase may have been due to the release of carbon dioxide produced as a result of earlier activity and trapped in the soil. This explanation would account for the brief minor increase in carbon dioxide production obtained for the unoiled soils.

Much of the released carbon dioxide would however have presumably been lost to the atmosphere during the 2 h the chambers were left open to atmosphere following

remixing. A further possibility was that remixing the soil resulted in a better distribution of water throughout the soil volume. The periodic addition of water evenly to the soil surface to maintain the soil moisture content may have resulted in a soil moisture gradient in the soil column. Under such conditions water may have become limiting towards the soil base during the course of the experiment. The response may also have been due in part to improved soil aeration which would have resulted from remixing.

The immediate stimulation following the second application of oil in contrast to the lag phase which followed the first was considered evidence that the soil contained microbial species capable of decomposing hydrocarbons. Maximum weight of oil mineralized from 67 d to 106 d was obtained for soil amended with nutrients and two 10 percent applications of oil. Highest average monthly rate of oil mineralization as a percentage of that added was again greatest for soil containing 5 percent oil and amended with nutrients. A maximum of 1.22 g of oil/ 100 g soil or 24.1 percent of that added was mineralized over the 106 d experimental period for the same soil.

V. GAS CHROMATOGRAPHIC ANALYSIS OF OIL EXTRACTED FROM TIMARU SILT LOAM

(1) Preliminary Experiments

Before attempting to determine changes in the amount of oil present in Timaru soil it was first necessary to

devise a suitable method by which oil could be extracted and analysed quantitatively. These therefore were the aims of the following experiments. Fifty g of 2 mm sieved air-dried Timaru soil was moistened to 50 percent of W.H.C. and mixed thoroughly with 2.5 g $\pm$ 0.005 of 'Vitrea 22'oil. The soil was then oven-dried at 80°C for 16 h and ground using a mortar and pestle to pass a 500µm mesh sieve.

Ten ml of a solution containing 1.6 mg/ml of n-hexadecane in 'X4' solvent, an organic solvent comprising mainly hexane and pentane (Shell Oil New Zealand Ltd) was mixed thoroughly with 20 g of the prepared soil using a glass rod. A stream of compressed air passed across the soil was used to evaporate the solvent.

One hundred mg of spiked soil was weighed into a 10 ml stoppered glass tube and shaken with 1 ml of 'X4' solvent. The procedure was repeated twice (3 replicates) and the stoppered tubes allowed to stand for 12 h.

A glass syringe was used to inject 5 µl samples samples of soil extract and a standard containing 4.3 mg/ml of 'Vitrea 22' oil and 80 µg/ml of n-hexadecane into a Hewlett Packard 'Research' gas chromatograph (model 5751B) with a flame ionization detector. Following initial experiments with an OV 1 column, a 2.0 m x 3.2 mm (i.d.) stainless steel column packed with Dexil 300 on Chromosorb W acid washed 80-100 dimethyl dichlorosilane was used for the experiment with a nitrogen flow of 20 ml/min. The

oven temperature was programmed from 250°C to 320°C at 20°C/min and held at the upper limit for 10 min. Detector and injection ports were at 350°C and 290°C respectively.

Concentration of oil in the soil extracts was determined by the following formula:

$$\text{Wgt of oil in 100 mg soil (mg)} = \frac{4.3 \times \text{Peak height n-hexadecane in standard}}{\text{Peak height n-hexadecane in sample}} \times \frac{\text{Envelope area, oil in sample}}{\text{Envelope area, oil in standard}}$$

where 4.3 = concentration of oil in standard (mg/mℓ).

Results are shown in Table 61 and Figures 31 and 32 .

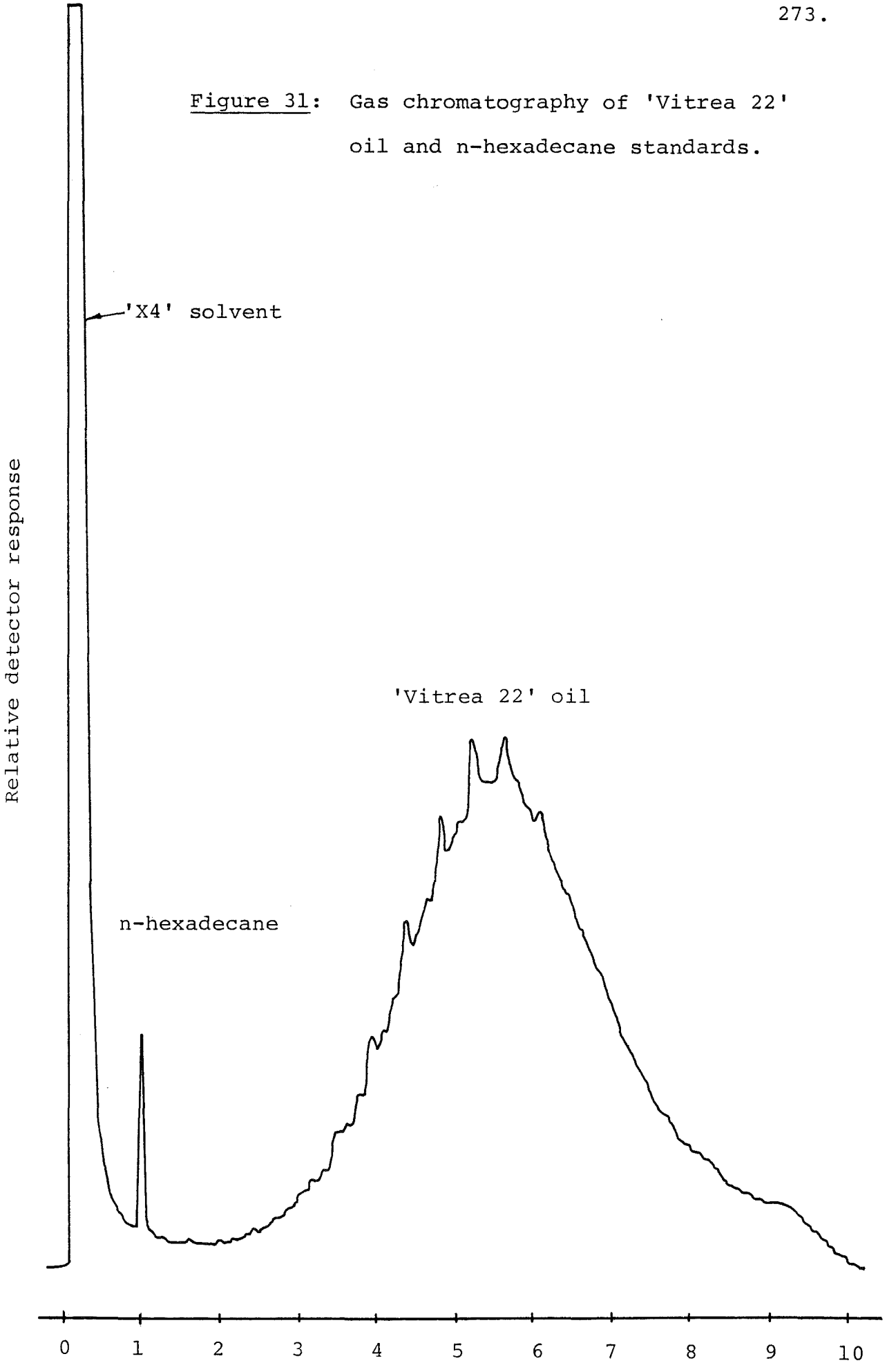
Table 61 : Extraction of 'Vitrea 22' oil from Timaru silt loam.

+Means of 3 replicates.

Sample	Concentration of oil in soil (mg/g)	Concentration of oil in extracts (mg/g)+	Percentage of oil recovered
1	50.0	49.5	99.0
2	"	51.6	103.2
3	"	49.3	98.6
$\bar{x}$		50.1	100.3
s		$\pm$1.3	$\pm$2.6

Mean amount of oil recovered was 100.3 percent. On the basis of these results the extraction method was judged satisfactory and was used for all subsequent oil analyses. The only modification was to the time for which the instrument was held at the upper temperature limit. This

Figure 31: Gas chromatography of 'Vitrea 22' oil and n-hexadecane standards.



(Chart recorder speed 1.5 cm/min)

was reduced from 10 min to 3 min when it was found that nothing further was detected once a temperature of 320°C had been reached.

(2) Gas Chromatographic Analysis of Oil Extracted from Timaru Silt Loam

Three replicates each of treatment and control soils prepared as described on page 271 were extracted at time zero and 106 d. A further set of samples was extracted after 66 d but time did not permit their analysis. Results are given in Table 62 and Figs 32-35.+ The amount of oil extracted from incubated oil treated soil decreased by approximately 50 percent or an average of 14 percent/month over the 106 d experimental period. Concentration of oil extracted from the control soil did not change significantly indicating that nonbiological decomposition of the oil was not significant and that the oil recovery rate had not changed. Oil not recovered from the treatment soil was therefore presumably sufficiently modified chemically to have rendered it insoluble in the extracting solvent. The data indicate that evaporative losses were negligible.

'Vitrea 22' has a carbon number range of C16 to C30, (Shell Oil N.Z. Ltd pers. comm. 1980). In a report by Supelco Ltd, a wax sample was chromatographed using a comparable column, C_{30} compounds were not retained by the column at temperatures greater than 280°C. On this basis it was concluded that the percentage of 'Vitrea 22'

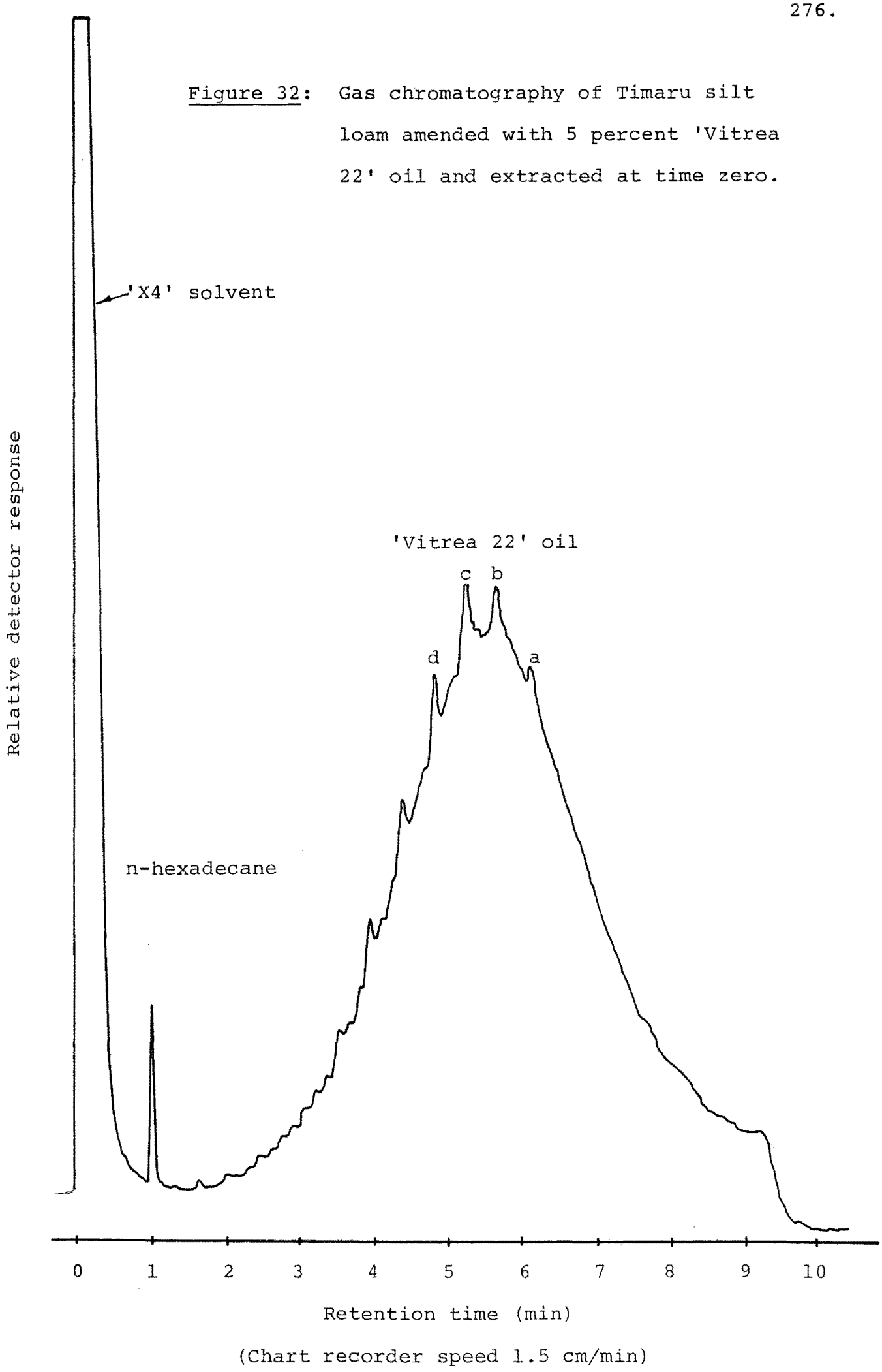
+ Chromatograms for unoiled and sterile oiled soils at time zero were similar to those shown in Figures 34 and 35 respectively.

Table 62: Loss of 'Vitrea 22' oil from extracts of Timaru silt loam after 106 d.

+Means of 3 replicates.

	Treatment		'Sterile' Control	
	Soil + 5 percent oil + nutrients	s	Soil + 5 percent oil + nutrients + $HgCl_2/CuSO_4$	s
Mean weight oil/100g air-dried soil at 0 d (g)+	4.72	±0.44	4.76	±0.24
Mean weight oil/100g air-dried soil after 106 d (g)+	2.34	±0.08	4.82	±0.26
Mean weight oil lost/100g air-dried soil/month (mg)+	667.29	-	-	-
Mean weight oil lost after 106 d as % of oil added+	50.42	-	-	-

Figure 32: Gas chromatography of Timaru silt loam amended with 5 percent 'Vitrea 22' oil and extracted at time zero.



(Chart recorder speed 1.5 cm/min)

Figure 33: Gas chromatography of Timaru silt loam amended with 5 percent 'Vitrea 22' oil and extracted after 106 d incubation.

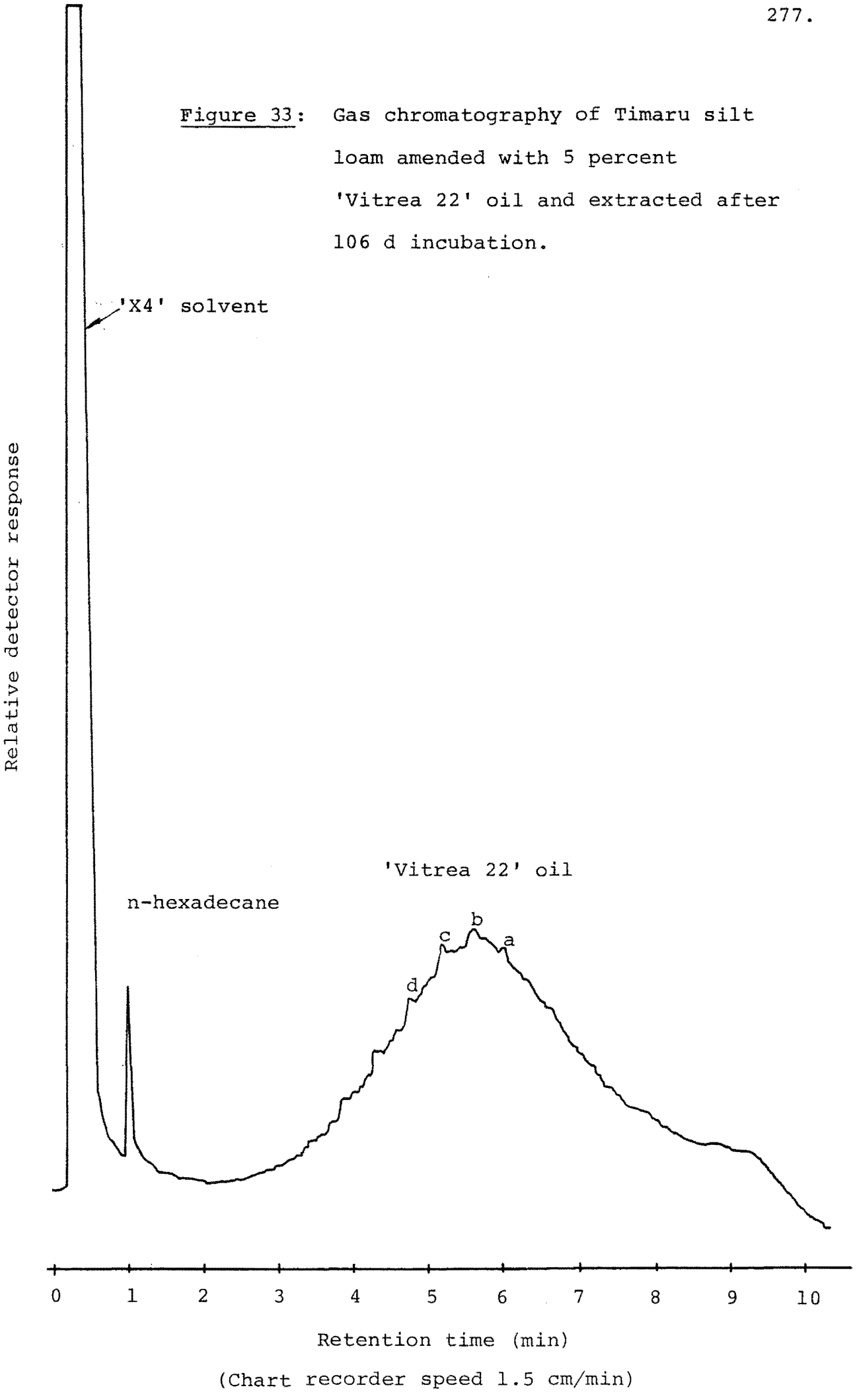


Figure 34: Gas chromatography of biologically inactive Timaru silt loam amended with 5 percent 'Vitrea 22' oil and extracted after 106 d incubation.

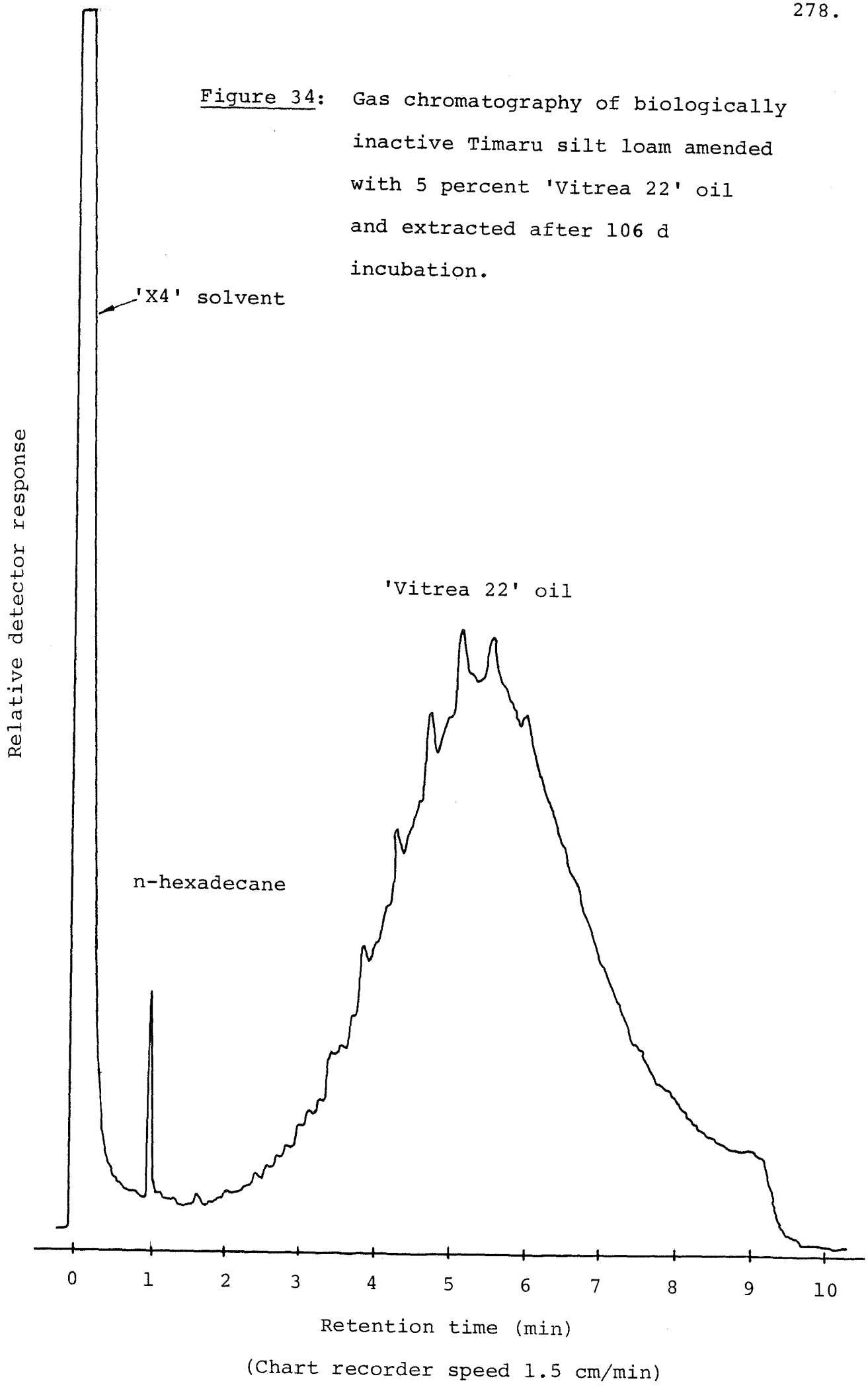
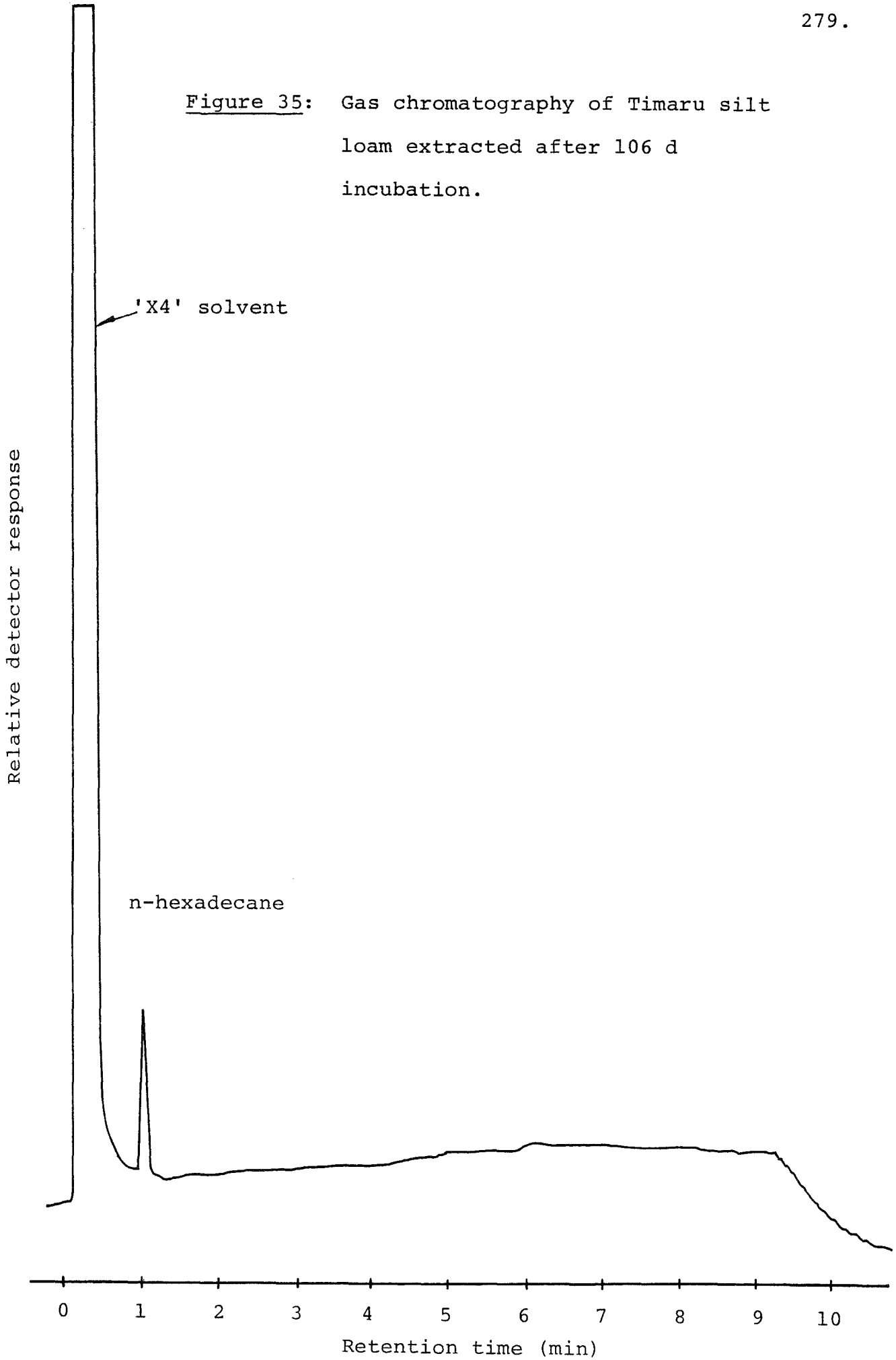


Figure 35: Gas chromatography of Timaru silt loam extracted after 106 d incubation.



(Chart recorder speed 1.5 cm/min)

oil not detected in the present investigation was negligible.

Comparison of chromatograms for oil extracted at time zero and after 106 d suggested changes in the relative concentration of at least some of the resolved peaks. Relative height of peaks a, b, c and d shown in Figures 32 and 33 changed from 1.00:1.18:1.19:1.01:0.76 for oil extracted at time zero to 1.00:1.09:1.02:0.79:0.56 for oil extracted after 106 d incubation. The pattern of change suggests that lower molecular weight hydrocarbon may have been more rapidly decomposed than those of higher molecular weight.

VI. THE EFFECT OF FRESH AND WASTE AUTOMOTIVE LUBRICATING OILS AND 'VITREA 22' LUBRICATING OIL ON CARBON DIOXIDE EVOLVED BY TIMARU SILT LOAM

Results obtained for the previous experiment provide evidence that micro-organisms were primarily responsible for the observed disappearance of 'Vitrea 22' oil from Timaru soil.

As described elsewhere, (p 48) waste lubricating oil, in contrast to 'Vitrea 22' contains in addition to paraffins, minor amounts of other hydrocarbon types and various additives together with a significant amount of petrol derived lead. There is evidence to suggest that alicyclic and aromatic hydrocarbons are less readily decomposed than aliphatic compounds (p 81) and that heavy metals such as lead may prove toxic to soil micro-organisms (p32). It was therefore considered likely that waste lubricating oil would be decomposed more slowly than 'Vitrea 22' oil.

A respiration experiment was therefore carried out to test the validity of this hypothesis. Waste automotive lubricating oil obtained from the Dominion Oil Re-refining Company Ltd, Auckland, New Zealand was mixed with remoistened air-dried Timaru soil at a rate of 5 percent (a.d.s.b.) as described for previous experiments. Lead content of the oil was 0.34 percent by weight. At this concentration the lead would have been added to the soil at a rate of approximately 170 µg/g. 'Vitrea 22' oil was applied at a rate of 5 percent by weight (a.d.s.b.) to a second amount of remoistened soil.

To simulate the original composition of the waste automotive lubricating oil, fresh oil was mixed with a further sample of remoistened soil (5 percent by weight a.d.s.b.). The fresh oil comprised engine and transmission oils mixed in the ratio 7.5:1.0, the proportions present in waste lubricating oil, (Wrightcars N.Z. Ltd pers. comm. 1980). Engine and transmission oils supplied by five major New Zealand supply companies, Shell Oil N.Z. Ltd, British Petroleum N.Z. Ltd, Mobil Oil N.Z. Ltd, Caltex N.Z. Ltd and Europa Oil N.Z. Ltd were used in equal amounts. The oils contained only trace amounts of lead (Shell Oil Co. N.Z. Ltd pers. comm. 1980). Soil treated with $HgCl_2/CuSO_4$ as earlier described served as a control. pH of the oil amended soils was 6.45; that of the unamended soil, 7.00. Treatment and control soils were replicated twice (three replicates). Other experimental details were as for previous respiration experiments. Weight of carbon dioxide evolved

over the 31 d experimental period was computed for treatment and control soils. Results were analysed using a one way anova, and are given in Table 63 and Figure 36 .

Table 63: Carbon dioxide evolved by Timaru silt loam amended with 'Vitrea 22' and fresh and waste automotive oils. +Means of 3 replicates. Incubation temp., 19°C.

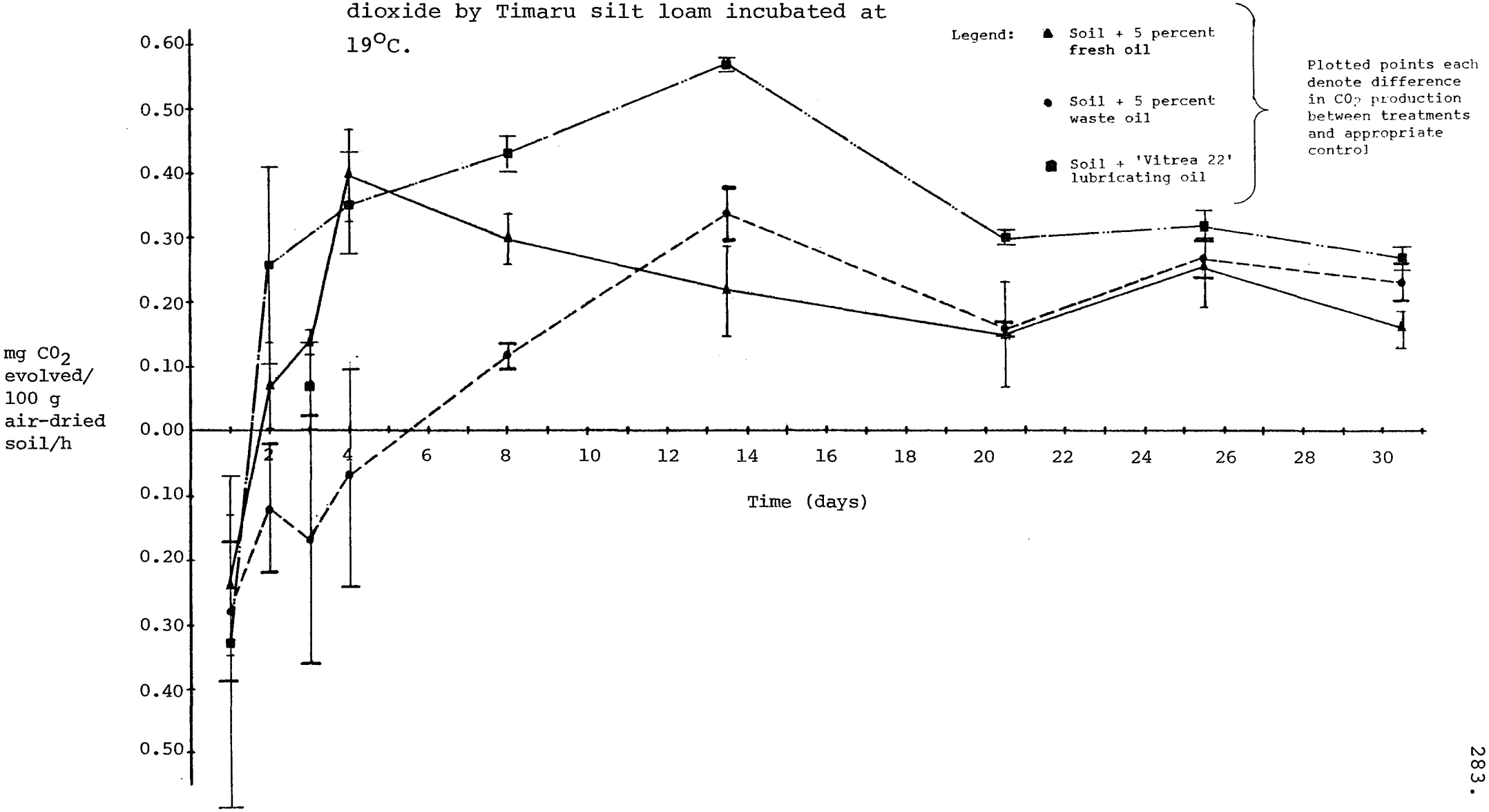
	Treatments			Controls	
	Soil + 5% fresh oil	Soil + 5% Waste oil	Soil + 5% 'Vitrea 22' oil	Soil only	Soil + $HcCl_2$ and $CuSO_4$
Total CO_2 evolved/ 100 g air-dried soil over 31 d+ (mg)	636.83	612.81	771.22	444.48	4.11

S.E. = ± 13.22 L.S.D. (5%) = 43.61

Table 64: Mineralization of lubricating oils in Timaru silt loam. +Means of 3 replicates. Incubation temp., 19°C.

	Oil Type		
	Fresh	Waste	Vitrea 22
Maximum weight of oil mineralized/100g air-dried soil after 31 d (mg)+	52.13	45.62	88.55
Maximum weight of oil mineralized as % of oil added	2.44	2.13	4.14

Figure 36: Effect of fresh and waste automotive lubricating oils and 'Vitrea 22' lubricating oil on evolution of carbon dioxide by Timaru silt loam incubated at 19°C.



Maximum weights of mineralized oil were also determined and are shown in Table 64 . Calculations were based on assumptions given on p259. Carbon dioxide production over 31 d was approximately 25 percent higher for soil treated with 'Vitrea 22' oil compared with that obtained for soils amended with fresh and waste automotive oil. Activity of all oil amended soils was initially slightly less or little greater than that obtained for unoiled soil but the lag phase was longer and production during it less for soil treated with waste oil.

Results indicate that carbon dioxide evolution and by inference, oil mineralization was affected by oil composition. A different hydrocarbon content, the presence of additives or both may have been responsible for the reduced activity of soil treated with automotive oils. The prolonged lag phase in the presence of waste oil may have resulted from the enhanced concentrations of soil lead.

Maximum mean carbon dioxide production rate obtained for 'Vitrea 22' amended soil was less than that obtained for similarly treated soil in a previous experiment (p 266). A possible explanation concerns the time interval between drying and rewetting of soil. Experiments by Soulides and Allison (1961) showed that carbon dioxide evolution by soil rewetted 10 d after drying was 40 percent greater than that of the control and only 20 percent for that remoistened 24 h after drying. The pattern of carbon dioxide evolution was consistent with that obtained for the present

investigation. Soil used for the above experiment was air-dried and used soon after its collection from the field. That used for the previous experiments was stored for 2-4 weeks at room temperature before use and would therefore have lost some water prior to having been air-dried.

VII. THE EFFECT OF LEAD ON CARBON DIOXIDE EVOLUTION BY TIMARU SILT LOAM AMENDED WITH 'VITREA 22' OIL

The effects of lead on soil biological processes have been reviewed elsewhere, (p 30). Results obtained for the previous experiment suggested that lead at a concentration of 170 µg/g may have been responsible for the prolonged respiratory lag phase obtained for Timaru soil treated with waste lubricating oil. Lead content of oil applied to the Timaru field plots was considerably higher (1.58 percent by weight) and concentration of added lead would have been approximately 1000 µg Pb/g soil for an equivalent oil application rate.

On the basis of results obtained for lead treated soils amended with a single alkane, n-hexadecane and fuel oil, a complex mixture of hydrocarbons, Jensen surmized that the activity of hydrocarbon degrading micro-organisms which decompose the alkane fraction of oils would not be inhibited by the addition of lead. Many such micro-organisms can use only a small range of substrates (p 22) and it was therefore conceivable that for soil amended with a large variety of alkanes such as is present in 'Vitrea 22' oil,

rate of decomposition of some hydrocarbons would be reduced. A further experiment was therefore carried out to test the validity of these hypotheses.

Lead occurs in waste lubricating oil as lead halides, lead oxy-halides and lead oxy-sulphates. The occurrence of particular lead compounds depends upon their melting points in relation to local surface temperatures. (Shell Oil Company N.Z. pers. comm. 1973a).

Lead as $PbCl_2$ was used for the present investigation because of its relatively high solubility and ready availability. Lead chloride was dissolved in deionized water to provide solutions containing 136 mg/ℓ and 272 mg/ℓ of lead. Each solution was mixed thoroughly with 50 g of 2 mm air-dried Timaru soil to provide a moisture content 52 percent of W.H.C. Lead concentrations of the amended soils were 500 and 1000 µg/g respectively. To test for possible osmotic effects, two 50 g samples of Timaru soil were amended with potassium chloride to give chloride ion concentrations the same as those of the lead amended soils. A further 50 g of soil received only water. Each soil was mixed with 'Vitrea 22' oil at a rate of 5 percent by weight (a.d.s.b.). Fifty g of air-dried soil treated with $HgCl_2$ and $CuSO_4$ as earlier described served as a control. Soil pH values were as follows: soil only, 6.95; soil + 5% oil + 500 µg/g Pb, 6.35; KCl_{500} control, 6.40; soil + 5% oil + 1000 µg/g Pb, 6.25; KCl_{1000} control, 6.40. Treatment and control soils were replicated twice (3 replicates). Other

experimental details were as for previous experiments.

Mean rates of carbon dioxide production and total carbon dioxide produced over the 32 d experimental period were determined for treatment and KCl control soils. Results were analysed using a one way analysis of variance and are shown in Figure 37 and Table 65 respectively.

Total carbon dioxide released over 32 d was approximately the same for all the soils and mean rates of respiration obtained for the lead amended soils were not significantly different from those of their respective KCl controls.

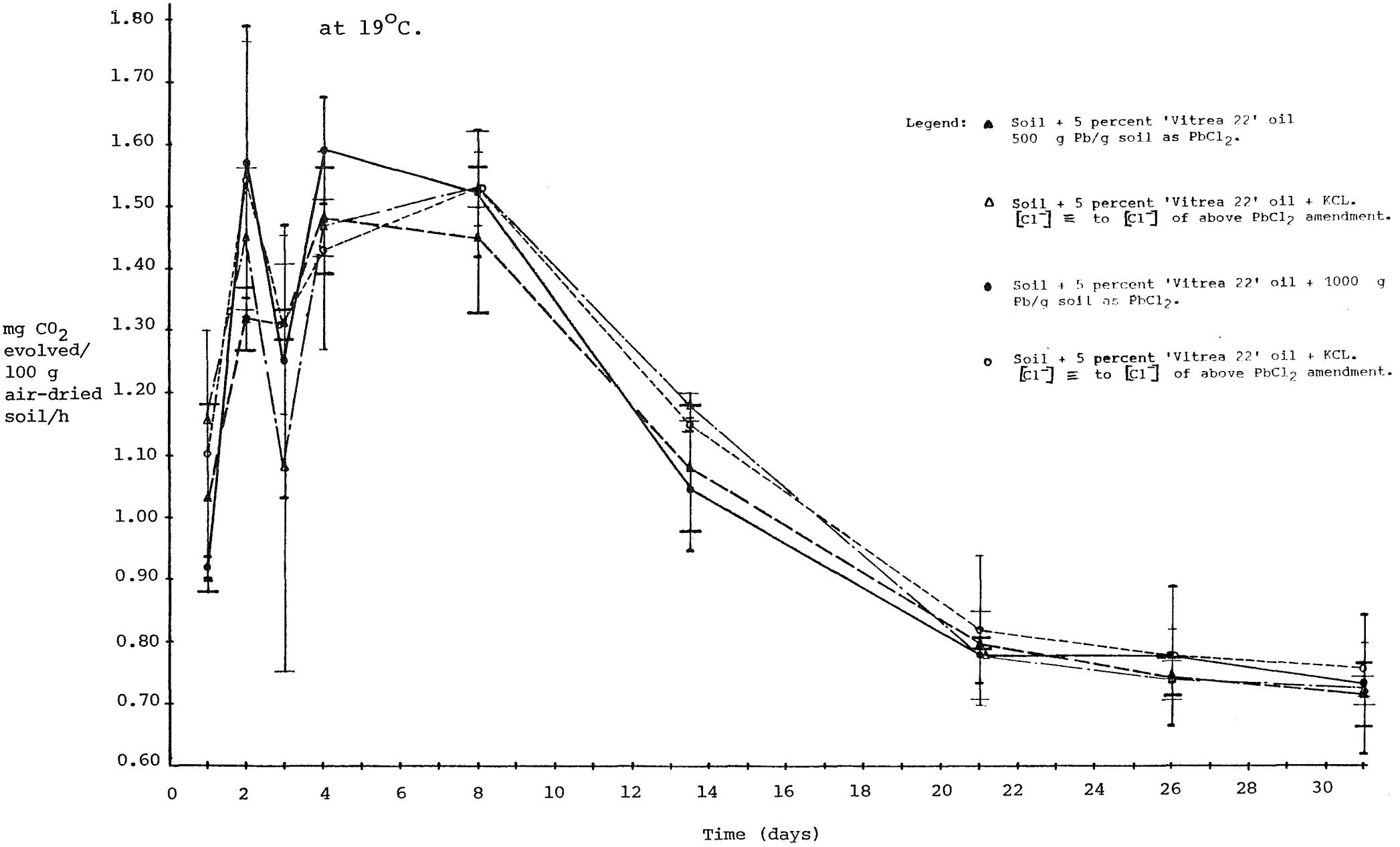
The results indicate that addition of lead at concentrations of 500 µg/g and 1000 µg/g had no significant effect on carbon dioxide produced by oil treated Timaru soil and were in agreement with the hypothesis of Jensen (1977) p 30. They also suggest that some factor other than lead was responsible for the prolonged and accentuated 'lag phase' obtained for Timaru soil treated with waste lubricating oil.

Table65 : Carbon dioxide evolved by oiled Timaru silt loam amended with lead and incubated at 19°C. +Means of 3 replicates.

	Soil + Pb 500 $\mu g.g^{-1}$	Soil + Oil + KCl Control	Soil + Pb 1000 $\mu g.g^{-1}$	Soil + Oil +KCl Control	Soil + Oil	Soil + $HgCl_2/CuSO_4$
Carbon dioxide evolved/100 g air-dried soil over 32 d (mg)+	715.69	731.73	730.14	742.51	746.36	4.14

$F_{(3,8)} = 0.615$ n.s.

Figure 37: Effect of lead on evolution of carbon dioxide by Timaru silt loam amended with 'Vitrea 22' oil and incubated at 19°C.



VIII. THE EFFECT OF 'VITREA 22' OIL ON EVOLUTION OF CARBON DIOXIDE BY TIMARU AND TAKAHIWAI SILT LOAMS INCUBATED AT 19°C.

Results obtained for previous experiments indicated that the amount of carbon dioxide evolved by Timaru silt loam was increased by the addition of 'Vitrea 22' oil. The experiments also provided evidence that the increased activity was due primarily to decomposition of the added oil. Soils relatively rich in organic matter contain a more diverse microflora than those which have a low organic matter content, (Pelczar 1958). As discussed elsewhere (p 22) many hydrocarbon decomposers have quite specific substrate requirements and it was therefore conceivable that oil would be degraded more rapidly by a more diverse soil microflora. Furthermore, the larger microbial population size present in an organic rich soil might be expected to result in a more rapid rate of oil decomposition. Timaru silt loam has a relatively low organic matter content, (approximately 5 percent), and an experiment was therefore carried out to compare the amounts of carbon dioxide evolved by Timaru silt loam treated with oil and a similarly amended soil relatively rich in organic matter.

Takahiwai silt loam was chosen because it is relatively rich in organic matter, texturally similar to Timaru silt loam and formed in a similar type of coastal environment.

A spade was used to randomly sample approximately 5 kg of Takahiwai silt loam top soil (top 15-18 cm) from

a site near Karaka Point (grid reference N47/364350). The top soil comprises a dark reddish brown friable peaty silt. A_{11} horizon (0-5 cm) of weak fine granular structure underlain by 15 cm of black silt in having a moderate medium blocky structure. New Zealand D.S.I.R. Soil Bureau - Soil Survey Report 33, (1977). pH, W.H.C., total carbon, total nitrogen and the carbon: nitrogen ratio for both soils are shown in Table 66. Total soil carbon was analysed by ashing 5g over-dried soil samples at 400°C for 16 h and total nitrogen by the semi-micro Kjeldahl method.

Sieved (2 mm) air-dried Takahiwai silt loam was moistened to a water content 50 percent of water holding capacity and mixed thoroughly with 'Vitrea 22' oil at a rate of 5 percent (a.d.s.b.). Fifty g of soil on an air-dried basis was weighed into a respiration chamber and spread evenly over the bottom. A second respiration chamber contained similarly treated Timaru soil. The procedure was repeated for both soils except that oil was omitted. Soil of each type treated with $HgCl_2$ and $CuSO_4$ as previously described served as a control. Treatment and control soils were replicated twice (3 replicates). Other experimental details were as described for previous experiments.

Total carbon dioxide evolved over 31 d way computed for treatment and control soils and compared using a two way analysis of variance. Results are given in Tables 67 & 68.

Table 66: Physical and chemical characteristics of Timaru and Takahiwai silt loams.

Soil Type	pH, soil: water 1:2.5	Water holding capacity as % of oven dried soil	Total carbon as a % of oven-dried soil	Total Nitrogen	C:N
Takahiwai silt loam	7.05	193	15.99	0.58	27.6
Timaru silt loam	6.95	95	5.4	0.17	31.7

Table 67 : Carbon dioxide evolved by Timaru and Takahiwai silt loams amended with 'Vitrea 22' oil. $^{+}$Means of 3 replicates. Incubation temp., 19^{o}C.

	Soil Type	Soil + 5% 'Vitrea 22' Oil	Soil Only	Soil + $HgCl_2$/ $CuSO_4$
Total CO_2 evolved/ 100g air-dried soil over 31 d (mg)$^{+}$	Takahiwai silt loam	923.23	641.72	4.17
	Timaru silt loam	716.61	458.77	4.08

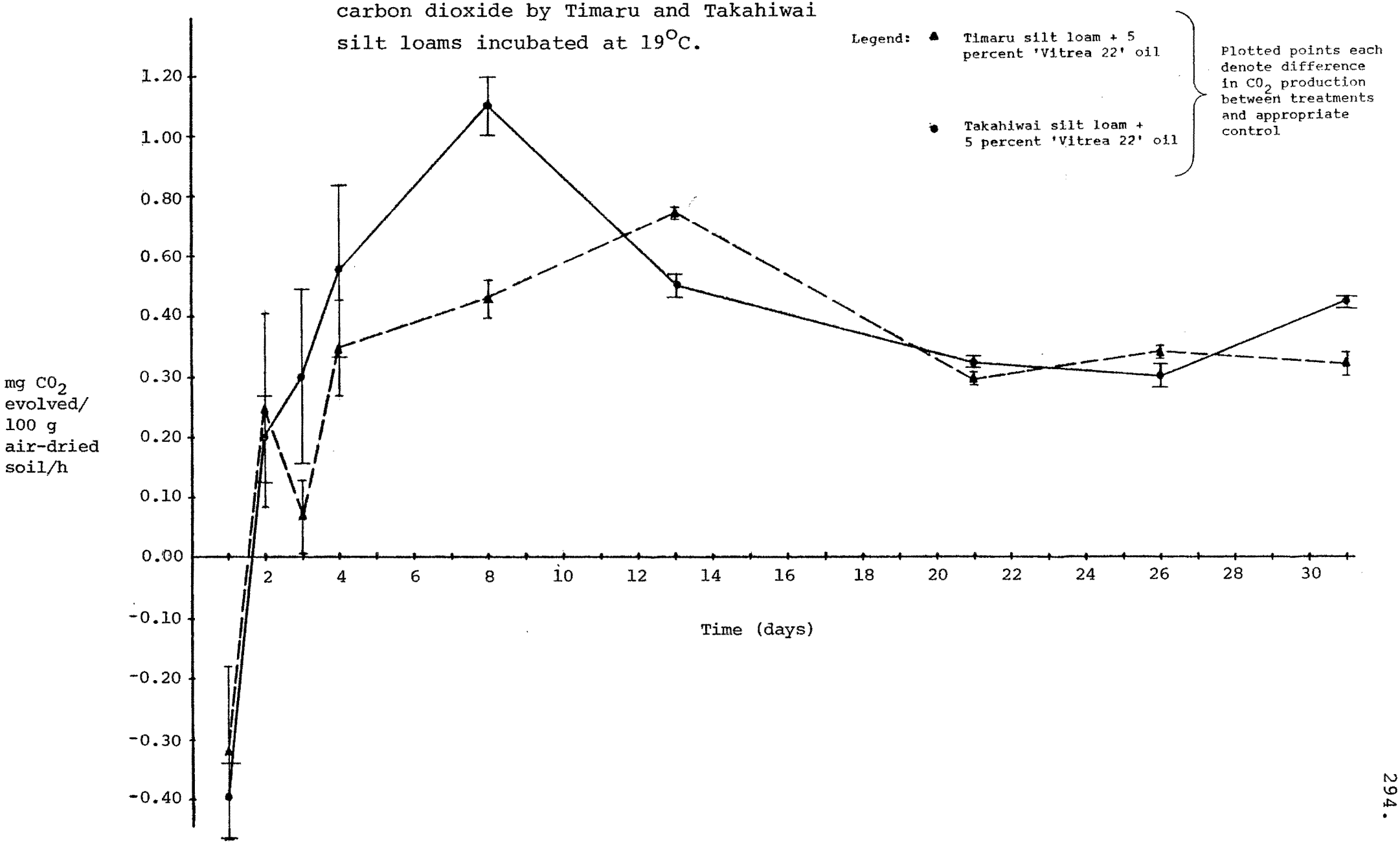
S.E. = ± 3.7150 L.S.D. (5%) = 13.74

Table 68 : Mineralization of 'Vitrea 22' oil in Timaru and Takahiwai silt loams over 31 d. $^{+}$Means of 3 replicates.

Soil Type	Maximum weight oil mineralized/100g air-dried soil over 31d mg^{+}
Takahiwai silt loam	89.92
Timaru silt loam	82.36

t = 3.86*

Figure 38: Effect of 'Vitrea 22' oil on evolution of carbon dioxide by Timaru and Takahiwai silt loams incubated at 19°C.



The results indicate that activity of the unamended Takahiwai soil was approximately 40 percent greater than that obtained for Timaru soil. Maximum weight of mineralized oil for Takahiwai soil was however only 10 percent greater than that obtained for Timaru soil over the 31 d experimental period. Inspection of Figure 38 indicated that much of the increased activity obtained for Takahiwai soil occurred during the first 10 d of the experiment before carbon dioxide production rates had stabilized. Amounts of oil mineralized over the period 21 d to 31 d, shown in Table 69 were not significantly different and it was therefore concluded that oil mineralization was not significantly different for the two soils.

Table 69 : Mineralization of 'Vitrea 22' oil in Timaru and Takahiwai silt loams over 21 d to 31 d. $^{+}$Means of 3 replicates.

Soil Type	Maximum weight of oil mineralized/100g air-dried soil over 31 d mg^{+}
Takahiwai silt loam	81.35
Timaru silt loam	77.50

t = 1.84 n.s.

CHAPTER VII

DISCUSSION

Maximum average rate of waste lubricating oil disappearance of Timaru silt loam was of the order of 9.1 t/ha/month and obtained for a 224 t/ha oil application rate and tillage at level 3. This result was lower than that observed by Kincannon *et al.* (1972) (35.7 t/ha/month for an application rate of 1029 t/ha oily sludge) and higher than the 6 t/ha/month obtained by Raymond *et al.* (1976) for approximately 30 t/ha of waste lubricating oil. Field and laboratory evidence from the Timaru experiment suggested that oil losses due to surface runoff, sub-soil leaching and evaporation were small and that oil recovery rate was constant for the duration of the field experiment. Average rates of oil loss based on gravimetric method and obtained for the 25 treatments were therefore assumed to require no adjustment. A similar claim made by Kincannon *et al.* for sub-soil leaching was not confirmed by his data which for a sub-soil depth of 0.3 m showed an increase in extractable oil of up to 63 percent over the 17 month period of his experiment. Description of the top soil (58 percent sand 14 percent silt and 28 percent clay) as sandy clay and at a depth of 0.61 m from the surface, loam and at 1.22 m, sand, suggested that increases of this magnitude should not have been discounted but more samples taken at greater and lesser depths to

determine its real extent.

Results from a pilot sampling programme indicated that sixty soil samples of approximately 400 g randomly drawn from each field plot of 11.7 m^2 were necessary to obtain consistent data from the present field experiment and permit a satisfactory statistical analysis. Inconsistencies in the soil extract data of Kincannon et al. (1976) were probably due to an inadequate sampling programme. (Kincannon sampled three systematically selected points for each soil plot of 1500 m^2 and Raymond, 5 randomly selected sampling points for each plot of 5.1 m^2. Plots of both unduplicated experiments were systematically arranged and details of sample size not given).

Average rate of disappearance of waste lubricating oil from soil in the present field investigation was determined by the rate at which it became unavailable to the extracting solvent. Evidence of the likely nature and extent of hydrocarbon alteration was provided by microbiological, soil and plant growth experiments.

Increased microbial activity of the oiled soils was indicated by large increases in microbial numbers compared to unoiled control soil. Bacteria isolated from oiled Timaru field plots caused extensive physical alteration of pure paraffinic oil in liquid culture and because they were able to grow and reproduce (as determined by large increases in numbers), presumably altered it chemically.

Increased carbon dioxide production from Timaru silt loam amended with pure paraffinic oil under laboratory conditions was further evidence for increased microbial activity. The pattern of production for soil treated with nutrients and repeat applications of oil compared with that of unoiled control soils was evidence that at least part of the observed increase in carbon dioxide production was due to decomposition of the added oil rather than mineralization of soil organic matter. A corresponding decrease in the amount and apparent altered composition of oil present in the treated soil compared with that obtained for biologically unactive soil as determined by gas chromatographic analysis of soil extracts, was considered to indicate that the oil had been modified chemically by biological processes.

A maximum of 1.22 g oil/100 g of nutrient amended soil or 24.4 percent of that added was mineralized over 15 weeks. Approximately 50 percent of the added oil was recovered from the soil after a corresponding period. The difference was therefore presumed to have been due to intermediate metabolic products which were either insoluble in the extracting solvent or not detected by the gas chromatographic technique used.

The estimated percentage oil mineralized, 24.4 percent, was approximately twice that obtained by Loynachan (1978) for crude oil added to Bodenburg silt loam at a rate of 6 percent and incubated at 10^{o}C for 115 d. The Bodenburg

soil was amended with nitrogen and phosphorus. Sterile controls were not included and changes in the concentration of oil in the soil were not determined.

Atlas & Bartha (1972) used CO_2 evolution and gas chromatography to follow the degradation of Swedish crude oil added at a rate of 1 percent (W/V) to sea water amended with nitrogen and phosphorus. Over 18 d an estimated 42 percent of the oil was converted to carbon dioxide and 70 percent degraded on the basis of gas chromatographic analysis of water extracts, proportions similar to those obtained for the present investigation. Recovery rate for oil extracted from the sea water was not given and the amount of oil assumed to have been degraded as determined from gas chromatographic analysis may therefore have been in error. Furthermore, water samples were extracted for analysis only at the completion of the experiment and it was therefore not be possible to ascertain extent of possible loss due to nonbiological processes.

Carbon dioxide production from Timaru soil treated with pure paraffinic oil was approximately twice that obtained for chemically more complex waste automotive lubricating oil. Approximately equal production from waste and chemically similar fresh automotive oil was considered evidence that the difference was due to the types of hydrocarbons present, the additive content of the oil or both, rather than the relatively small concentration of lead present. Total acid number of the waste lubricating

oil used was 3.06 mg KOH/g oil. Fresh automotive crankcase oils in comparison had an alkalinity equivalent to approximately 4 mg KOH/g soil (Shell Oil N.Z. Ltd., 1980). The approximately equal production obtained from the two chemically comparable oils was therefore considered evidence that the different pH of the oils had not significantly affected the inferred rates of oil mineralization.

Relatively low concentrations of exchangeable NO_3, NO_2 and NH_3 and evidence for phosphorus immobilization were further evidence of increased microbial activity in Timaru oiled soils. Near normal shoot growth of Tama ryegrass on the oiled soils 18 months after oil application under field conditions indicated that the biological properties of the oiled soil had been mostly restored. Moisture retention capacity of the oiled soils was similar to that of the control except at very low pressures (0.3 and 1.0 bar).

Both Kincannon et al. (1972) and Raymond et al. (1976) also used the rate at which oil became available in an extracting solvent to measure the rate of disappearance of oil from soil but claimed that rate of disappearance was synonymous with "decomposition" and "degradation" and "biodegradation" respectively, terms which they did not define. Kincannon et al. (1972) claimed that "microbial action" was responsible for the disappearance of oil from soil but micro-organisms isolated from the oiled soil were not shown able to use hydrocarbons as a sole carbon source. Bacteria and fungi isolated from oiled field plots by

Raymond et al. (1976) were found able to use pure hydrocarbons as a sole carbon source (p 113). Chemical evidence cited by Kincannon to indicate the extent of hydrocarbon "decomposition" included changes in the percentage composition of hydrocarbon classes (also studied by Raymond) and a decrease in long chain paraffins and of high boiling compounds in soil extracts from successive samplings. The validity of such evidence assumed the recovery rate for oil from soil was known and that it remained unchanged throughout the experiment. Neither investigation considered this aspect.

Average rate of soil disappearance (over 18 months) under field conditions increased with increased oil application rate. The pattern was repeated for rates of oil mineralization inferred for pure paraffinic oil mixed with Timaru soil both in the presence and absence of added nutrients. One possible explanation for the increase was that larger amounts of oil were accessible to the hydrocarbon degrading micro-organisms at higher oil application rates. Increased microbial activity which, assuming nutrients were not limiting, would have resulted from the higher temperatures observed for darker soils of higher application rate, may also have been partly responsible for the observed increased rate of oil disappearance under field conditions.

Loynachan (1978) obtained the same pattern of carbon dioxide production for crude oil mixed with Bodenburg silt loam at rates of 3 and 6 percent and incubated at 10^{o}C.

A similar increase in carbon dioxide production with oil application rate was obtained for Prudhoe Bay soil but only in the presence of added nutrients.

The patterns of respiration rates and oil disappearance obtained for the present investigations were also in agreement with the results of Kincannon et al. (1972) who claimed "about equal" rates of "decomposition" for three sludges of different chemical composition mixed with soil in the field but oil application rates, (as determined from increases in oil content of the soil after each of two oil applications) were considerably different (499, 1029 and 678 t/ha) thereby invalidating any comparison and were more closely related to application rate, a result similar to that obtained for the present study.

An increase in the average rate of oil disappearance after the application of nitrogen (11 months after oil application) and the lower exchangeable NO_3, NO_2 and NH_3 content of the oiled soils at the conclusion of the field experiment (and after the shoots of two Tama ryegrass crops had been harvested), was evidence that nitrogen was used by micro-organisms. Application of the nutrient coincided with the beginning of the warmer summer season and with a change in the method of cultivation which together may have obscured any effect. Use of phosphorus by soil micro-organisms was suggested by its immobilization in the oiled soils.

Further evidence for increased rates of oil decomposition in the presence of added nutrients was

obtained from soil respiration experiments. Inferred maximum average rates of oil mineralization of pure paraffinic oil mixed with Timaru soil amended with nitrogen and phosphorus were increased by 87 and 47 percent for 5 and 10 percent oil application rates over the first 66 d of the experiment. A larger increase, 70 percent, was obtained for nutrient amended soils with a 10 percent application rate following a further application of oil. This result is in contrast to a reduction of approximately 12 percent for comparable soils with a 5 percent application of oil.

The amount of 'plant available' phosphorus at the conclusion of the field experiment was found to have increased for soils of high oil application rate. Application of oil to the soil may have increased the amount of soil phosphorus available to plants through the lowering of pH or promotion of reducing conditions as suggested by Ellis et al. (1961) (p 36) or phosphorus, which occurs, in unused lubricating oil in organic combination may have been altered during its life in automobile engines or subsequently in the soil.

Further research would be required to determine relative importance, and the time and rate of nutrient application on the rate of disappearance of waste lubricating oil.

Kincannon et al. (1972) investigated the effect of fertilizer on the rate of oil disappearance from soil under field conditions and found average "decomposition"

rates (for the three oily sludge types) of 12.5, 24.0 and 25.5 t/ha/month for zero, moderately and heavily fertilized plots. Fertilizer including nitrogen as urea and ammonium nitrate, phosphorus as calcium hydrogen phosphate and potassium as potash ($K_2 0$) were applied at rates totalling 4.84 t/ha (N) 6.23 t/ha (P) and 0.98 t/ha (K) for "heavily fertilized" plots and 2.23 t/ha (N), 1.62 t/ha (P) and 0.49 t/ha (K) for "moderately fertilized" plots. Results were claimed by Kincannon to indicate a "direct" relationship between quantity of fertilizer and oil removal rate. Anomalously low "decomposition" rates he considered due to fertilizer excesses but gave no evidence to substantiate this claim. Inspection of Kincannon's data showed that for a 2 fold increase in applied nitrogen and 4 fold of phosphorus, an increase of only 5 percent in the average soil "decomposition" rate was obtained and that there was a close relationship between the rate of oil disappearance and oil application rate further confirming results of the present study. Kincannon's data for total soil phosphorus indicated losses of up to 85 percent for unfertilized oiled plots over the 17 months of field trial operation. No explanation was suggested for Kincannon's results but such high losses would seem unusual where microbial activity was held responsible for observed losses of oil from soil. A very small amount of phosphorus occurred in the oily sludges (average 7 ppm) and it was therefore likely that phosphorus in time would have become limiting. Results from the same study indicated that NO_3 and NH_3

concentrations for the unfertilized and fertilized oily soil after 17 months were low but lack of control plots made it difficult to conclude as to the possible fate of the nitrogen. Raymond et al. (1976) investigated the effect of fertilizer on the rate of oil disappearance and found an increase for fertilized plots after 40-50 percent of the oil had disappeared. However, the increase coincided with the onset of the warmer summer season when increased microbial and chemical activity might have been expected. Inconsistencies in oil application rate for fertilized and unfertilized plots also made it difficult to assess the quantitative effect of fertilizer, (nitrogen, phosphorus and potassium were applied but insufficient information was given to establish elemental application rates).

Results for the present study indicated that cultivation of the field plots increased the rate of disappearance of waste lubricating oil at all application rates and that there was an interaction between oil application rate and duration of cultivation. The benefit of extensive cultivation was most marked for 56 and 224 t/ha oil application rates. A possible explanation for the results might be that at the highest oil application rate, aeration may have been of continual importance while extended cultivation of the 56 t/ha plots may have increased accessibility of residual hydrocarbons to micro-organisms. Oil losses also occurred from uncultivated plots of all oil application rates. Cultivated plots continued to show a decreased oil concentration 58 weeks after oil application

(sampling 5) and therefore base concentrations could not be determined (base concentration was defined as that concentration below which no further significant oil loss occurred. Longer term investigation would be needed to determine any base and reason(s) for it. Cultivation did not result in a reduced rate of oil loss from the soil which suggested that moisture remaining in the soil was adequate for chemical and biological activity. Both Kincannon et al. (1972) and Raymond et al. (1976) assumed cultivation necessary to promote the disappearance of oil from soil. In Kincannon's study all plots received 2 ploughings/month for the first three months after which the frequency was increased in an attempt to increase the rate of oil "decomposition" while Raymond tilled all plots once a month with a rototiller. Where the object of an investigation was to establish the economic feasibility of a land disposal system, it was surprising that the effect of cultivation was not investigated. The cost/benefit of cultivation at Timaru was an important aspect of this study and is considered in Chapter VIII.

The effect of oil reapplication on the disappearance of oil from soil was not studied under field conditions during the present investigation. Comparison of soil respiration rates obtained for soils which received a single oil application with those which received two, provided evidence that oil mineralization rates were increased by reapplication. Perhaps this was not surprising considering the evidence that repeat oil applications were made to soil which contained micro-organisms

with the enzymes necessary to decompose hydrocarbons. Maximum increase in the inferred rate of oil mineralization of 35 percent was obtained for soil amended with nutrients and a 10 percent application of oil.

In contrast to all other oil/nutrient treatment combinations, a reduced rate of oil mineralization was obtained for soil with 5 percent oil and added nutrients following reapplication. One possible explanation for the pattern of results concerns the accumulation of intermediate products of oil decomposition. As discussed on p 20 there is evidence that water soluble fatty acids, intermediate products of oil decomposition may, in sufficient concentration, reduce or inhibit further oil decomposition, Bell (1971). Maximum inferred weight of oil mineralized during the first 66 d of the present experiment as a percentage of that added, (11.1) was obtained for the same treatment combination. As such, concentration of remaining oil would have been lowest for those soils. Assuming a ratio of 2:1 (p298) for the relative amounts of oil lost from the soil as determined from soil extracts and that mineralized, concentration of the unaltered oil remaining in the soil after 66 d would not have exceeded 3.88 percent and was probably less. Possibly the reduction in the amount of oil accessible to the soil micro-organisms was sufficient to have stimulated decomposition of intermediate products of oil degradation. Accumulation of water soluble fatty acids of shorter carbon chain length may have been sufficient to

have adversely affected rates of mineralization following oil reapplication.

In order to conduct a preliminary test of the hypothesis, respiration rates of soils incubated in open ended columns and intermittently flushed with water to remove water soluble fatty acids, could be compared with that of undisturbed soil columns. Before testing the hypothesis however it would be desirable to repeat the experiment for a longer period of time to confirm the pattern of results. Under field conditions, fatty acids would probably be leached from the soil and therefore be unlikely to accumulate in significant amounts.

Carbon dioxide production and by inference, oil decomposition was not significantly affected by the addition of lead as $PbCl_2$ rates up to 1000 µg Pb/g soil to Timaru silt loam mixed with 5 percent by weight 'Vitrea 22' paraffinic oil. The results were not inconsistent with the suggestion of Jensen (1977) that the decomposition of a complex mixture of alkanes would not be adversely affected by lead added at concentrations up to 5000 µg Pb/g soil. Jensen's suggestion was based on his finding that carbon dioxide production from soil amended with fuel oil, a complex mixture of hydrocarbon types including alkanes was affected by the addition of lead at a concentration of 5000 µg/g whilst that obtained for soil amended with a single alkane, n-hexadecane was not. On the basis of his results he surmized that the presence of lead had selectively inhibited micro-organisms which

decomposed fractions of fuel oil other than alkanes.

Since waste lubricating oil is largely of paraffinic composition, results obtained for experiments with paraffinic 'Vitrea 22' oil were considered evidence that decomposition of waste lubricating oil under field conditions would not have been adversely affected by the lead content of the oil. Further research would be needed to confirm or otherwise this extrapolation and to determine the effects of lead on the rate of disappearance of repeated applications of oil.

Lead analysis of shoots of Tama ryegrass grown on Timaru soil approximately 20 months after oil application to field plots indicated increased lead accumulation compared to that of the control. Maximum increase was obtained for 56 t/ha oiled plots while for 224 t/ha soil there was no significant increase, suggesting that the higher concentration of oil residues may have suppressed lead uptake. Further research would be needed to investigate this possibility and to determine whether uptake would increase with time. Experiments carried out to ascertain an acceptable method of lead analysis should be properly established before attempting to determine the lead concentration of the plant material.

Similar rates of carbon dioxide production obtained for oil treated Takahiwai and Timaru silt loams, soils of widely different organic content but similar C:N ratio and pH, suggested that soil organic matter did not significantly affect rate of oil mineralization.

The above field experiments indicated that under project conditions, loss of large amounts of applied waste lubricating oil did occur from Timaru soil over 18 months and that the rate of disappearance in economic terms was affected by both rate of oil application and tillage duration. Oil loss from sub-soil, surface runoff water and evaporative sources were not significant and diminishing soil extract weights over 18 months suggested that the waste lubricating oil was sufficiently altered chemically to render it insoluble in the extracting solvent. Biological properties of the oiled soils as indicated by shoot dry matter yield of *Lolium multiflorum* Lam. var. Westerwolds "Grasslands Tama" were almost completely restored after 20 months further suggesting biochemical/chemical alteration of the oil. Microbiological evidence and evidence for nitrogen and phosphorus immobilization in the oiled soils suggested that soil bacteria were at least partially responsible for modification of the hydrocarbons. Further evidence was provided by large increases in $C0_2$ production and a corresponding decrease in the oil concentration of biologically active soil amended with a mixture of paraffinic hydrocarbons. Unchanged oil concentration of biologically inactive soil indicated that the observed loss of oil was due primarily to biological processes. Increased soil respiration rates suggested that the rate of oil decomposition could be increased by the addition of nutrients increased oil application rate and by further applications of oil.

Evidence from other soil respiration experiments suggested that the rate of disappearance of waste lubricating oil from Timaru soil was not severely affected by the lead content of the oil. Lead uptake by the shoot system of *Lolium multiflorum* increased on the oiled soils.

CHAPTER VIII

COSTS AND RECOMMENDATIONS

The cost of waste lubricating oil disposal by land spreading will depend upon physical, chemical and biological properties of the soil and geographic location of the chosen site, amount of waste oil for disposal/unit area/unit time, cost of land, transport, fertilizer and labour. Results for rates of oil disappearance from the Timaru land disposal system gave some idea of costs under project conditions. The effect of waste lubricating oil application rate and number of cultivations on the cost of oil disposal is shown in Figure 39.

Cultivation cost at February 1981 was $28/h or $140/ha for a single rotary cultivation.

Other costs (February 1981) include:

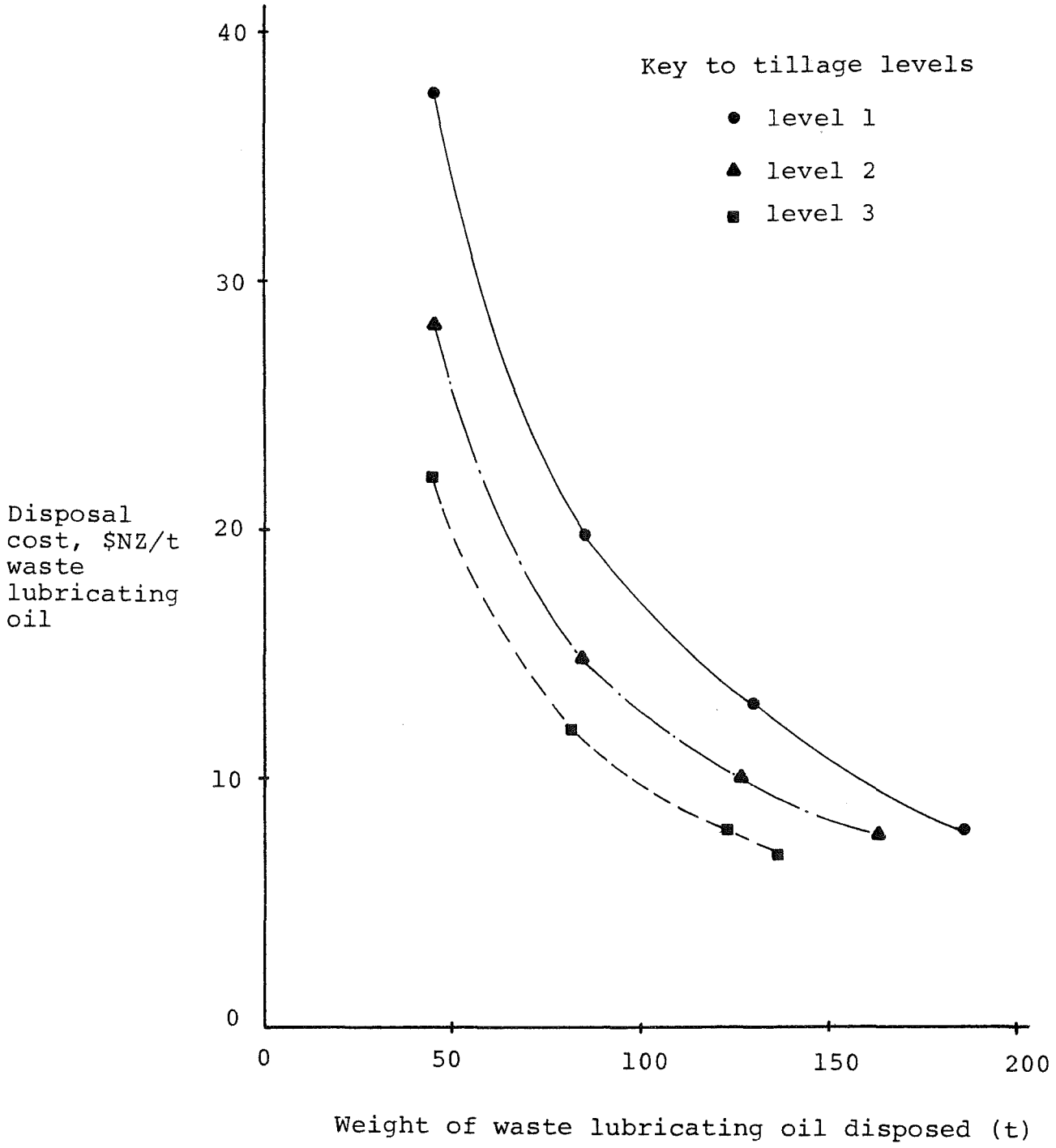
Lime:	$N.Z.
at an application rate of 10 t/ha, cost/ha	= 80.00

Application rate is determined by pH of the unoiled soil and reapplication for subsequent oil applications may be necessary.

Superphosphate:	
cost/tonne of waste lubricating oil for disposal	= 0.32

Experimental results suggest that the application rate of 753 kg/ha (based on an oil application rate of 224 t/ha) was excessive.

Figure 39: Effect of tillage duration on cost of waste lubricating oil disposal.



$N.Z.

Calcium Ammonium Nitrate:

cost/t of waste lubricating oil for disposal
(based on an oil application rate of 224 t/ha) = 0.35

Spreading Costs:

(based on a distance of 9 km from contractor to site).

lime and superphosphate applied together/t oil = 0.07

calcium ammonium nitrate/t oil = 0.07

waste lubricating oil/t = 8.00

The following recommendations are intended as guidelines to increase the rate of disappearance of waste lubricating oil from soil with minimum cost and environmental hazard.

1. The site chosen for waste oil disposal should be planar with a slight grade to facilitate water runoff, have an impermeable sub-soil, not be shaded from the sun and where possible not be located near water supplies for animal or human consumption (surface or underground).

2. Lime should be applied to and mixed with the soil prior to oil application. Rate of application to the cultivated site should be determined from the soil pH.

3. Rates of waste lubricating oil application and reapplication should be determined by the oil holding capacity of the top-soil.

4. Drainage channels should be provided to collect surface runoff water which should preferably be discharged over an adjacent area of land.

5. Regular checks should be made for the presence of oil and lead in sub-soil,runoff waters and any nearby aquifers. Hydrocarbons of particular importance are those considered to have carcinogenic properties including benz-3, 4-pyrene which occurs in used lubricating oil at a concentration of 0-10 ppm. Data for benz-3, 4-pyrene concentration in top and sub-soil and surface runoff water were not determined for the Timaru field trial because its measurement was considered to be outside the scope of this thesis. Oil application rates should be modified where loss of hydrocarbons or lead is considered to exceed limits considered safe by health authorities.

6. Nitrogen and phosphorus are important microbial nutrients, requirements for which will vary with respect to site and rate of waste oil application. A practical guide to application rates might be a C:N:P ratio of 50:15:1 (assuming waste lubricating oil to have a general formula of C_nH_{2n}. Phosphorus (as superphosphate) may be applied to and mixed with the soil prior to oil application. Results of the above experiments suggest a single application to be sufficient for several waste oil applications. Nitrogen application should be delayed (to reduce losses due to denitrification) until after several cultivations

and the onset of the drier, spring/summer months when the soil should be less anaerobic.

7. The disappearance of waste lubricating oil from soil is promoted by cultivation which improves aeration and should therefore be most frequent in the first few months after oil application. Subsequent cultivation programmes will be determined by the amount of waste lubricating oil/ unit area/unit time available for disposal and cost. Soil micro-organisms require moisture and cultivation should not be repeated to the extent that drying out of the soil results. Water-logging should also be avoided. Routine checks on oil concentration of the soil would provide an additional guide to frequency and duration which can best be achieved by use of a rotary cultivator.

8. It is possible that after several applications of waste lubricating oil, capacity of the site for further disposal may become limited by a build-up of metals and other toxic residues when a further site would be required for oil disposal. Any reduction in this capacity would be indicated by repeated measurements of oil concentration of the soil.

9. Further research concerning (a) the possible uptake of lead and hydrocarbons by the shoot systems of a wide range of plants and (b) the accumulation of heavy metals and carcinogenic hydrocarbons in the top soil would be necessary before deciding on the future use of a land disposal site.

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APPENDIX 1

Analysis of Timaru Waste Lubricating Oil †

Closed Flash Point	92°C
Viscosity Kinematic cSt @ 98.9°C	17.2
Total Acid Number (ASTM D664)	4.5 mg KOH/g
	Percent weight
Lead	1.58
Zinc	0.11
Barium	nill
Calcium	0.14
Phosphorus	0.046
	p.p.m.
Potassium	5.0
Chromium	7.5
Iron	300.0
Aluminium	12.0
Nickel	0.9

† Supplied by the Shell Oil New Zealand Ltd.

APPENDIX 2

Morphological characteristics and profiles of bacteria isolated from oiled Timaru silt loam

Isolate A1:

Agar colonies: Circular whitish smooth, raised, glistening.

Profile:

Mobility	+	Glucose	A
Growth in air	+	Lactose	-
Catalase	+	Sucrose	-
Oxidase	-	Mannite	-
O/F test	0	Growth on McCon	+
ONPG	-	Nitrate	-
Arginine	-	Gelatin hydrol.	-
Lysine	-	Malonate	+
Ornithine	-	Gluconate	-
Citrate	+	Phenylalanine	-
H_2S	-	KCN	+
Urease	-		
Indole	-		
VP	-		
MR	-		

Isolate B1

Agar colonies: greyish, irregular, smarming.

Profile:

Mobility	+	Glucose	A
Growth in air	+	Lactose	-
Catalase	+	Sucrose	-
Oxidase	-	Mannite	A
O/F test	F	Growth on McCon.	+
ONPG	-	Nitrate	-
Arginine	-	Gelatin hydrol.	-
Lysine	-	Malonate	-
Ornithine	-	Gluconate	+
Citrate	-	Phenylalanine	+
H_2S	-	KCN	+
Urease	-(W)		
Indole	-		
VP	-		
MR	+		

Presumptive classification - a Proteus species. A considerably extended range of biochemical tests would be needed to confirm this classification.

APPENDIX 3

Composition of Plant Nutrient Solution

Constituent element	p.p.m.
N	448
P	31
K	156
Mg	48
Ca	80
S	160
Fe	3.0
Cl	0.68
B	0.5
Mn	0.5
Zn	0.05
Cu	0.02
Mo	0.02

APPENDIX 4

Chemical Analysis of Auckland Waste Lubricating oil[+]

Total Acid Number (ASTM D664)	3.06 mg KOH/g
	Percent Weight
Lead	0.34
Zinc	0.069
Calcium	0.090
Phosphorus	0.099
	p.p.m.
Potassium	12.5
Chromium	7.0
Iron	225.0
Aluminium	36.0
Nickel	2.0
Barium	38.0

[+]Supplied by Shell Oil New Zealand Ltd.

ERRATA

Page	
108	Table 19 - sampling 3 for '6' read '4'.
113	Line 12 - omit 'oiled'.
192	Line 1 - should read 'suggest deficiency....'
193	Line 3 - should read '....was claimed to be 4.51. Application .....'
203	Line 8 - should read '....Appendix 1 Results are given on to 226'
238	'2' outside square brackets should be omitted. Footnote: 'An excess of hydroxyl ions was present throughout the experiment.'
244	Line 25 For '23' read '24'
262	Line 12 - for 'p258' read 'p259'
270	For line 7 read 'surface' for 'base'
280	Line 5 should read '....peaks a, b, c, d and e ....' Line 7 - for '0.56' read 0.65'
291	Line 9 - for 'over' read 'oven'
292	Table 66 - for column 'Total Nitrogen' read 'Total Nitrogen as a % of oven-dried soil'
300	Line 3 for 'soil' read 'oil'
302	Line 12 should read '....and losses were .....'